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Steric hindrance and its effect on chromosome movement in *Vicia faba* somatic cells

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Summary. The arrangement of chromosome arms in metaphases and anaphases has been studied in *Vicia faba* root meristem cells. During metaphase, the long chromosome arms are aligned parallel to the spindle axis. As a consequence, at the onset of anaphase, one chromatid can move straight ahead to the spindle pole whereas the other has to invert its orientation. Specially in narrow cells it has been observed frequently that some chromatids move in a reverse orientation to the pole, i.e., they move telomere-first instead of centromere-first. This behaviour results in a chromatid which protrudes beyond the main group of late anaphase or telophase chromatids. It is discussed that the most likely explanation for the phenomenon is that in narrow cells chromatid behaviour is influenced by steric hindrance by the tightly packed surrounding chromatids and microtubules. When there is insufficient room, some chromatids are unable to make the required U-turn. Under such conditions the kinetochore of a non-inverted chromatid pulls the chromatid in a reverse orientation to the pole. An alternative explanation, i.e., protruding chromatids being the result of a neocentric activity at the telomere end of a reverse-directed chromatid or the lateral associations of spindle microtubules, failed to find support by electron microscopical studies.

Keywords: Anaphase; Chromosome movement; Protruding chromatids; Steric hindrance; *Vicia faba*.

Introduction

At the metaphase stage of mitosis, all centromeres are aligned in the equatorial plane. The orientation of the chromosome arms is apparently dependent on the length of the chromosomes in relation to the size of the metaphase plate. In most plant species, relatively small chromosomes lie more or less parallel to the

equatorial plane, whereas relatively large chromosomes tend to be arranged parallel to the spindle axis (see, for example, the cine-micrographic studies by Bajer and Molé-Bajer 1953, 1954, 1956). During anaphase, chromatids that are aligned parallel to the equatorial plane move in a direction perpendicular to their metaphase orientation (Fig. 1 A). With respect to large chromosomes, which are aligned parallel to the spindle axis, the situation is somewhat different. At metaphase, both chromatids of one chromosome arm face the same pole. As a consequence of this positioning, one chromatid arm must invert itself during anaphase, whereas the sister chromatid arm can move directly to the opposing pole without any change in orientation (Fig. 1 B). In the classical literature about mitosis, Bělař (1930) gives a detailed description of this phenomenon in stamen cells of *Tradescantia virginiana*.

Such reorientation of the chromatid arms is a process which requires certain space. In continuation of a study of mitosis in *Vicia faba* root meristem cells with a reduced cell height (Oud and Nanninga 1992), we now have focussed the attention to cells with a severely reduced width (defined as the side-to-side distance of a cell, perpendicular to the spindle axis; further called "narrow" cells). In such cells, all chromosomes are closely aligned parallel to the spindle axis. We observed that in these narrow cells it is sometimes impossible for a chromatid to make a U-turn during early anaphase, apparently because of the limited space. Such close confinement leads to steric hindrance, which influences anaphase chromatid

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Fig. 1. Simplified 2-D scheme of mitotic chromatid separation in a wide (**A** and **B**) and in a narrow (**C**) cell, with small (**A**) and large (**B** and **C**) chromosomes. In the case of small chromosomes both the centromeres and chromosome arms are aligned in the metaphase equatorial plane, whereas long arms are aligned parallel to the spindle fibres (dotted lines). Note the necessity for a 180° change in orientation for half of the long chromatids during early anaphase, and note the creation of protruding chromatids (arrows in **C**) during anaphase because space limitations in narrow cells, preventing a 180° rotation, result in a reverse movement of the chromatids. For the sake of simplicity only one arm is drawn for each chromosome

behaviour. In narrow cells we found chromatids which move to the pole telomere-first (see early anaphase in Fig. 1 C). Since all kinetochores move over nearly the same distance from the equatorial plane to the spindle pole, a chromatid which failed to turn will, by the end of anaphase, protrude beyond the rest of the complement (late anaphase in Fig. 1 C). On the basis of the conventional, confocal and electron microscopical studies presented in this paper, we argue that a reverse chromatid movement (i.e., telomere first) is a process that achieves orderly separation of chromatids in narrow cells.

Materials and methods

Conventional light microscopy

Lateral roots from 10-day-old *Vicia faba* plants grown in flower pots were fixed in 3 : 1 absolute methanol : glacial acetic acid (v/v). These roots were macerated and stained according to the Feulgen method. Some slides were left unsquashed, sealed with rubber cement and examined directly under the light microscope. Other Feulgen stained roots were squashed, whereafter the coverslips were removed by liquid nitrogen and then mounted in Depex. In addition to the above described procedure, other lateral roots were fixed in Navashins fixative, embedded in paraffin wax, sectioned and stained with Heidenhain's haematoxylin according to Johansen (1940).

Confocal microscopy

For these studies primary roots of germinating *Vicia faba* seeds were used. To increase the percentage of cells in meta- and anaphase, 3-day-old seedlings were incubated for 18 h in 2.5 mM hydroxyurea (which prevented the cells from entering S-phase) in Hoagland's solution at 24°C (Doležel et al. 1992). Thereafter, the roots were allowed to grow for another 15 h in the absence of hydroxyurea, and fixed in 3 : 1 absolute methanol : glacial acetic acid (v/v). The fixed

roots were macerated and stained according to the Feulgen method, transferred to 45% acetic acid (v/v), whereafter unsquashed, nail polish sealed preparations were made. The preparations were analysed with a Leica confocal microscope, using the 514 nm line of the argon laser for fluorescence excitation. Depending on the image, voxel sizes were used of 100 to 200 nm lateral, and 330 to 500 nm axial. The 3-D images consisted of 24 to 38 optical sections. For the analysis and visualisation of the confocal images a HP 425T workstation and Scilimage software (Kate et al. 1990) was used. The stereo pairs shown in Fig. 2 have to be viewed with uncrossed eyes.

Electron microscopy

Approximately 2 mm of tissue was cut from each bean root tip and placed into 3% glutaraldehyde with 2% formaldehyde in 0.1 M phosphate buffer (pH 7.2, room temperature). After a series of buffer washes and osmic acid fixation, the root tips were embedded in 100% Polarbed 812 resin. Longitudinal sections were cut 5 μm thick, mounted on to glass slides, stained with 0.05% toluidine blue and studied under a light microscope to locate and photograph cells of interest. Selected sections were re-embedded in fresh resin and re-sectioned at 90 nm thickness for EM examination and mounted on copper grids. These sections were double stained with saturated uranyl acetate in 50% ethanol, followed by lead citrate (Venable and Coggeshall 1965). Serial sections were studied and photographed using a Philips 201C TEM.

Results

Cell and chromosome size

Cells in the root tip of *Vicia faba* vary in their anatomy. A range of cell shapes and sizes exist in the meristem. Some cells are cubical and are relatively wide, whereas others are comparatively long and narrow. In this study we focused on metaphase and anaphase chromosome behaviour in relation to cell width. It proved to be relevant, as described below, to make a biparti-

Fig. 2. Confocal images of Feulgen stained *Vicia faba* root cells. **a** Metaphase; the line in the background indicates the orientation of the equatorial plane. **b** Metaphase-anaphase transition in a cell with all chromosome arms parallel to each other and perpendicular to the equatorial plane. **c** Early anaphase in a wide cell (diameter: 33 μ m); arrows indicate chromatids which are inverting their orientation. **d** Early anaphase in a narrow cell (diameter 16 μ m); thin arrows indicate three chromatids which moved in a reverse orientation towards the pole (see also sections 7 and 10 + 11, and compare with Fig. 1 C); thick arrow indicates chromatid which initially moved in a reverse orientation, whereafter it started to invert its orientation (same chromatid as shown in section 5), whereas in section 17 two chromatids are indicated which started to rotate at the onset of anaphase (all arrows in the individual sections point to centromere positions; *t* telomere; *equat.* approximate position of the equatorial plane). Compare the chromatids in these sections with Fig. 6. **e** Early and mid anaphase; arrows indicate centromere position in reverse oriented early anaphase chromatids; arrowheads indicate protruding mid anaphase chromatids which are the result of a reverse chromatid movement. **f** Late anaphase with two protruding chromatids (arrow-heads); between the two sets of chromatids the large satellites can be seen which are often the last parts to be fully separated. Bars: 10 μ m

tion with respect to the width of the cells. We defined narrow cells as being less than 15 μm in diameter in sectioned material, which corresponds to approximately 20 μm in unsquashed Feulgen preparations (HCl and HAc maceration causes a swelling of the cells). Cells with a diameter of at least 15 or 20 μm , respectively, are defined as wide cells.

The *V. faba* karyotype has a diploid number of twelve chromosomes, comprising two metacentric (M) chromosomes, each approximately 18 μm in length at metaphase with a prominent satellite, and ten smaller sub-telocentric (S) chromosomes each between 10 and 12 μm long.

Metaphase chromosome arrangement

At metaphase, the chromosomes characteristically orient themselves with their centromeres at the equator, in preparation for separation of the sister chromatids (Fig. 2 a, b). In general, the chromosome arms show a lengthwise alignment with the spindle axis; the telomeres facing towards one or the other pole. Some arms curve and splay out radially (Fig. 2 a), or all virtually straight arms are arranged uniformly parallel to the spindle axis (Fig. 2 b). This difference in chromosome arrangement also can be seen in anaphase (compare the wide cell in Fig. 2 c and the narrow cell in Fig. 2 d). In many metaphases there is an unequal distribution of the chromosomes with respect to the orientation of the chromosome arms towards one or the other pole (Fig. 2 a, b).

Anaphase chromatid separation

At the transition of metaphase to anaphase the sister chromatids separate over their full length (Fig. 2 b), whereafter the sister chromatids begin their journeys to the opposite poles. In the case of chromosomes which are aligned parallel to the spindle axis, the centromere region of one of the chromatids of each chromosome inverts its orientation in a characteristic fashion. First, the movement of the centromere region of the “inverted” sub-telocentric chromatid results in a “J”-shaped arm, followed by a “U”, which progresses to another “J”, and finally the chromatid arm straightens (see Fig. 2 b–e). The two metacentric chromosomes show a comparable behaviour. There is a remarkable difference in a sharpness of the U-turn of the chromatids in wide and in narrow cells. In wide cells the distance between the proximal and distal part of a U-shaped chromatid arm is several micrometers,

whereas in narrow cells it is often less than 1 μm (compare the selected chromatids in Fig. 2 c and the chromatid in section 5 of Fig. 2 d).

Reverse movement of chromatids

In narrow cells at early anaphase some chromatids with straight long arms, project telomere-first towards their pole. Figure 2 d shows three of such reversed chromatids. To facilitate the interpretation of the orientation of some of the tightly packed chromatids in Fig. 2 d, four relevant optical sections are shown separately. The sections 7 and 10 + 11 show reversed chromatids. The centromere position of the subtelocentric *V. faba* anaphase chromatids is characterised by either a weakly stained region, or indirectly by the very small short arm which often is oriented perpendicular to the long arm. All arrows in the sections of Fig. 2 d point to centromere positions. In all three examples of reverse-oriented chromatids the distance from the equator to the telomere is greater than the length of the long arm. In the sections 10 + 11, for example, the distance from the equatorial plane to the telomeres of the two reversed chromatids is 15 μm , whereas the *V. faba* chromosome arms are only 10 to 12 μm long. In other words, these chromatids have shifted with the telomeres in advance of their centromere towards the pole.

Apart from the straight, reverse oriented chromatids mentioned above, also U-shaped chromatids are found where the telomeres are located further from the equator compared to their initial metaphase position. Examples can be seen in section 5 of Fig. 2 d and in the top half of the left anaphase in Fig. 2 e (compare with the J-shaped chromatids in section 17, whereby the telomeres have not shifted away from the equator). As above, the crucial point here is that some chromatids have moved several micrometers telomere-first towards the pole, indicating a reverse movement of the chromatids.

In the five confocal images of early anaphases with at least one protruding chromatid (cf. Fig. 2 d), we recorded the spatial position of the particular chromatid within the spindle. Since even in hydroxyurea pretreated roots the number of early anaphases is relatively small, the sample size for this analysis was restricted to twelve chromatids. No indication was found for a preferential position of the protruding chromatids, because also in narrow cells all chromatids are arranged in a ring, i.e., at the periphery of the spindle, leaving the central part free of chromatids

Fig. 3. *Vicia faba* late anaphase (A–D) and telophase (E) configurations with chromatids which protrude in a reverse orientation beyond a half-spindle. **A** Feulgen stained squash preparation (*c* centromere; *t* telomere). **B** Unstained and unsquashed HCl/HAc macerated root tip cell (phase contrast). **C** 5 µm thick section of a cell embedded in resin and stained with toluidine blue; this section has been used for further EM study, as can be seen in Fig. 5. **D** and **E** Haematoxylin stained paraffin sections (interference contrast). Bars: 10 µm

(till the end of prophase this area is occupied by the nucleolus).

Protruding chromatids at late anaphase and telophase

At the end of the anaphase stage the overall orientation of the chromatids is characterised by centromeres which all face the spindle pole, whereas the telomere ends of the chromatid arms are facing towards the cell plate. At this stage, however, as well as the subsequent telophase stage, chromatids can be observed which protrude in a reverse orientation from the main group of chromatids (Figs. 2 e, f and 3 A–E). These late anaphase protruding chromatids are either straight or bent, but never folded or coiled.

A light microscopic analysis of 706 late anaphases in squash preparations of *V. faba* root tips showed 63 complements (8.9%) with a chromatid arm extending telomere-first towards a pole (Fig. 3 A). This readily identifiable feature also has been observed in unsquashed and sectioned preparations for light microscopy (Fig. 3 B–E), in optical sections (confocal microscopy) of unsquashed material, and in series of ultra-thin sections analysed by electron microscopy (see below). Table 1 gives a survey of the number

and distribution of protruding chromatids as observed in late anaphase. The data show that approximately half of the cells in which the phenomenon occurs have one protruding chromatid, a quarter have two of them (either one in each half of an anaphase configuration, or both in the same half), and the remaining quarter show three or more protruding chromatids.

Another common feature is that protruding chromatids most frequently occur in narrow cells. To illustrate this, we have measured in paraffin sections (cf. Fig. 3 D) the width of 50 late anaphase cells: half of them had one or more (partly) protruding chromatids, the

Table 1. Number and frequency of protruding chromatids observed in the two halves of 63 late anaphase configurations

	1 ^a	1 ^b	2	2 ^c	2	3	3	3	3
	0	1	0	1	2	0	1	2	3
n	33	10	4	6	4	5	0	1	0
%	52.4	15.9	6.3	9.6	6.3	7.9	0	1.6	0

^a One protruding chromatid in one half, none in the other half; cf. Fig. 3 B

^b cf. Fig. 3 D

^c cf. Fig. 3 A

Fig. 4. Plots of the size of two sets of 25 late anaphase cells in sections of *Vicia faba* roots (as Fig. 3 D), with (▲) and without (○) one or more chromatids which protrude in a reverse orientation beyond a half-spindle (a), and the relation between the number of protruding chromatids/anaphase (same cells as in a) and the width of the cell (b). Note that all cells with protruding chromatid(s) are less than 16 μm wide

other half had none. The results are shown in Fig. 4. The mean width of the cells in the two samples is respectively 11.4 μm (s.d. = 2.33) and 16.1 μm (s.d. = 2.31). A statistical evaluation shows a significant difference between the two samples ($t = 7.12$; $p < 0.001$). In this material (root sections) protruding chromatids are observed in cells with a width of 15 μm or less. In confocal images of Feulgen stained material the width of cells with protruding chromatids never exceeded 20 μm . Figure 4 also shows the relation between cell width and the total number of protruding chromatids per anaphase. Anaphases with three or more protruding chromatids are found in the most narrow cells, whereas one or two protruding chromatids are observed in a wider range of cell sizes.

Microtubule orientation in anaphases with protruding chromatids

In addition to the light and confocal microscopical work, we included an electron microscopical analysis of microtubule orientation in relation to reverse-directed chromatids. This was done because in meiotic cells it has been shown that the reverse-oriented movement of chromosomes can be caused by microtubules attached to the telomere region ("neocentric activity"; see Discussion for details and references). To ascertain whether or not microtubules were involved in the process, either by attachment directly to the end of the chromosome or by lateral attachments, we examined 5 μm thin sections of several target cells identified by light microscopy (one of them is shown in Fig. 5 C).

For electron microscopy, these sections were re-embedded and re-sectioned in series of 90 nm sections. Serial sections directly through and adjacent to the telomere of 12 chromatids moving telomere-first

were examined in detail. As a positive control, Fig. 5 A and B shows an example of microtubules attached to the kinetochore of one of the kinetochore-first oriented chromatids. Neither in the series of sections of this target cell nor in series of other cells did we find any indication of microtubule attachment to the telomere end of a reverse-directed chromatid (Fig. 5 C and D shows a representative example). The microtubules which can be seen in the vicinity of the telomere of the protruding chromatid are flanking the chromatid.

Identification of protruding chromatids

Of the 63 anaphases with a protruding chromatid, 14 involved an M-chromosome and 49 an S-chromosome. A χ^2 test at the 5% level shows no significant variation between the M- and the S-chromosomes in reverse mobility. The data therefore indicate no difference in behaviour between the M- and S-chromosomes. Further chromosome identification within the group S-chromosomes was impossible because of too small differences in their morphology.

Discussion

The present study deals with the observation of anaphase chromatids which protrude, in a reverse orientation, beyond the half-spindle in *Vicia faba* root meristem cells. Extension of chromatids towards the mitotic pole similar to protruding chromatids observed in the current study has been noted in some older literature about the mitotic cycle in *Allium cepa* (Mäkinen 1963, and references therein). The author offered no direct explanation for the phenomenon, but suggested: "that the arms in question are passively attached to the chromosome-to-pole fibres or to the pole-to-pole fibres". Mäkinen avoided the term "neo-

Fig. 5. Anaphase cell of *Vicia faba* with a chromatid arm extended telomere-first towards the pole. The 5 μ m thick section of this target cell (shown in Fig. 3 C) was re-embedded, re-sectioned and double stained with uranyl acetate and lead citrate, and observed under electron microscopy. **A** and **C** Two different 90 nm sections. The box in **A**, magnified in **B**, encloses a centromere of a chromatid moving conventionally kinetochore-first; note the microtubules extending from the kinetochore region. **C** and **D** Telomere region of the reverse-directed chromatid identified in Fig. 3 C. Although microtubules surround the chromatid, none are attached to the telomere. *c* Centromere; *kr* kinetochore region; *t* telomere; *m* microtubule. **A** and **C**, $\times 3300$; **B** and **D**, $\times 65,000$

centric activity”, otherwise used by Bajer and Östergren (1961), with reference to transient movements by endosperm metaphase chromosome arms, usually in the direction towards the spindle equator.

Bajer and Östergren in fact felt uncomfortable with this term since it might give the reader the wrongful impression that new centromeres were being formed. However, they did not want to introduce a new term

Fig. 6. Schematic representation of anaphase chromatid movement in a narrow cell, and the effect of steric hindrance on the closely packed chromosomes which are aligned parallel to the spindle axis over almost their entire length (compare with the stereo images in Fig. 2). The sister chromatids of chromosome "a" show the usual behaviour, i.e., one moves straight ahead to the top pole, whereas the other inverts its orientation at early anaphase to move centromere-first to the bottom pole. Chromatid "b", however, is unable to rotate because of the limited space, therefore the kinetochore of "b" pulls the long arm of the chromatid in a reverse orientation to the pole. The movement of chromatid "c" starts like that of "b", but because of the separation of the two sets of chromatids there is enough space to rotate in mid anaphase. The thin lines represent the bundles of kinetochore microtubules (pole-to-pole microtubules are not drawn)

for the phenomenon. In contrast to mitotic chromosomes, the occurrence of neocentric activity is widely accepted as being an aberrant form of *meiotic chromosome* separation in rye, maize and other grass species (Lima-de-Faria 1983, Viinikka and Kavander 1986, Kavander and Viinikka 1987). Our EM studies do not give any evidence for the attachment of spindle fibres other than to the kinetochore regions of *V. faba* somatic chromosomes (Fig. 5 A–D). Microtubules can clearly be seen surrounding the arms of the protruding chromatids but none is attached to the chromatin in non-centromeric regions. This repeated observation is substantiated by recent immuno-fluorescent labelling experiments of microtubules in *V. faba* root sections by N. de Ruijter (pers. comm.).

Steric hindrance affects anaphase chromatid re-orientation

Because of the lack of evidence for the existence of neocentric activity, we have to consider another mechanism to explain protruding chromatids. Therefore two additional observations have to be taken into account. First, protruding chromatids only occur in narrow cells (see Fig. 4), and second, the necessity for one of the sister chromatids of each chromosome to invert its metaphase orientation during early anaphase

(see Fig. 6). The sister chromatids that have to make a 180° turn, must squeeze their way between the other chromatids and surrounding microtubules (see the chromatids which move to the bottom pole in Fig. 6). In organisms, like *V. faba*, with large chromosomes which occupy much of the volume in a meristematic cell, limitation of space becomes an important factor when considering the mechanics of anaphase chromatid movement. Close confinement of the tightly packed chromosomes and microtubules in a narrow cell, a feature we refer to as steric hindrance, may lead to difficulties in the re-orientation of chromatids. The tendency that the number of protruding chromatids is correlated with the width of the cell (as indicated in Fig. 4) is fully in accordance with the steric hindrance hypothesis. The observed unequal distribution of chromosome arms (most arms at one side of the equatorial plane) will increase steric hindrance.

Chromatids which cannot invert start to move in a reverse direction, with the kinetochore at the rear. A force is generated that is transmitted to the chromatid arm which begins to move to the pole telomere-first (chromatids "b" and "c" in Fig. 6; see also sections 7 and 10 + 11 of Fig. 2 d). Some chromatids are able to invert in mid anaphase (chromatid "c", which is comparable to the chromatid indicated in section 5 of Fig. 2 d), possibly because the separation of the two

groups of chromatids results in a reduction in the degree of steric hindrance.

To enable chromatid rotation, it is likely that there has to be an angle between the chromatid and the microtubules (see chromatid "a" in Fig. 6). Assuming that chromatids possess a certain stiffness, a U-turn is impossible if chromatids and microtubules are fully parallel to each other and closely aligned (see chromatid "b"). This situation occurs frequently in narrow cells. In wide cells, however, chromosome arms splay outwards towards the periphery of the cell, which makes chromatid rotation relatively easy (compare chromatid orientation in Fig. 2 c and d).

A chromatid which completely failed to invert is characterised at late anaphase and telophase as a structure which protrudes in a reverse orientation beyond the main group of chromatids (Figs. 2 e, f and 3 a–e and chromatid "b" in Fig. 6). The question arises: what factors are responsible for preventing the protruding chromatid from "collapsing" when there is no longer steric hindrance by surrounding chromatids. It may be that chromatids are more rigid than is generally considered. Stiffness of the chromatids is the main point Bělař (1930) mentions to explain his observation of chromatids which are "directed towards the pole", a phenomenon in *Tradescantia virginiana* stamen cells, similar to our protruding chromatids. Other factors may be physical constraints imposed by the surrounding pole-to-pole and kinetochore-to-pole microtubules, cytoplasmic streaming may influence chromatid alignment (Bajer and Molé-Bajer 1956, Kuroda 1990), or some unknown kinetic forces may align the chromatid in the direction to which it is moving, possibly comparable to the a-kinetochoric transport of chromosome fragments lacking a centromere (see review by Fuge 1990, and references therein), or the microtubule-mediated chromosome transport *away* from the pole (not involving kinetochores) as has been described for the paternal chromosomes of *Trichosia pubescens* in primary spermatocytes (Fuge 1994). Moreover, "polar ejection forces" by polar microtubules are an essential part in the so-called "smart kinetochore" model of mitotic chromosome congression (see review by Rieder and Salmon 1994). Although the latter two examples refer to movements away from the pole, it cannot yet be excluded that polar microtubules (not attached to kinetochores) also can be involved in a poleward movement. In this respect a new model for chromosome movement during mitosis has to be

mentioned, as recently proposed by Forer and Wilson (1994). This model supposes the existence of forces on chromosome arms that are separate from the pulling force at the kinetochore. In particular, the authors think that certain motor molecules – for example similar to kinesin – in the spindle matrix may produce forces which directly act on the chromosome arms. If such motor molecules contribute to the reverse movement of chromatids, then they can also act as "guidances" which prevent severe deformations of protruding chromatids.

The kinetochores of the chromatids which move telomere-first are pulling in reverse. In this respect we deliberately avoided the use of the word pushing, which might give rise to the idea that the motor forces of a pushing kinetochore are different to that of a pulling kinetochore. In our view the behaviour of a kinetochore in a reverse oriented chromatid is no different to the kinetochore which is leading (i.e., pulling) the chromatid arm to the pole. Moreover, the term "pushing kinetochores" has already been used to explain the oscillating movement of kinetochores towards and away from the pole, as repeatedly observed by others in living newt lung cells at anaphase (Skibbens et al. 1993 and references therein).

A combination of large genome size and limitations of cell space, as occurs in *V. faba* root tip meristems, gives rise to steric hindrance where physical constraint influences chromatid behaviour during anaphase separation. Reverse movement is seen only under special structural conditions, namely in narrow cells. When there is insufficient room for a chromatid to execute a turn, then the chromatid will move in reverse towards the pole.

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