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Ultraviolet microbeam irradiation of chromosomal spindle fibres in *Haemanthus katherinae* endosperm

I. Behaviour of the irradiated region

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SUMMARY

We used an ultraviolet microbeam to irradiate chromosomal spindle fibres in metaphase *Haemanthus* endosperm cells. An area of reduced birefringence (ARB) was formed at the position of the focussed ultraviolet light with all wavelengths we used (260, 270, 280, and 290 nm). The chromosomal spindle fibre regions (kinetochore microtubules) poleward from the ARBs were unstable: they shortened (from the ARB to the pole) either too fast for us to measure or at rates of about 40 μm per minute. The chromosomal spindle fibre regions (kinetochore microtubules) kinetochore-ward from the ARBs were stable: they did not change length for about 80 seconds, and then they increased in length at rates of about 0.7 μm per minute. The lengthening chromosomal spindle fibres sometimes grew in a direction different from that of the original chromosomal spindle fibre. The chromosome associated with the irradiated spindle fibre sometimes moved off the equator a few micrometers, towards the non-irradiated half-spindle.

We discuss our results in relation to other results in the literature and conclude that kinetochores and poles influence the behaviour of kinetochore microtubules.

Key words: *Haemanthus*, area of reduced birefringence (ARB), chromosomal spindle fibres, microtubules, ultraviolet light

INTRODUCTION

Areas of reduced birefringence (ARBs) on chromosomal spindle fibres were first produced after a microbeam of ultraviolet light was focussed onto the chromosomal spindle fibres of crane-fly spermatocytes (Forer, 1965, 1966). An ARB was a discrete region of reduced birefringence with edges formed by the apparently unaffected chromosomal spindle fibres outside the irradiated area. The nature of the ARB was initially unknown, but subsequent work showed that ARBs were regions in which kinetochore microtubules were absent (Leslie and Pickett-Heaps, 1983, 1984; Wilson and Forer, 1988; Snyder et al., 1991), and, therefore, that the ARB was delimited by the ends of severed kinetochore microtubules. The ends of the severed microtubules at the poleward edge of the ARB (the edge closest to the pole) are newly created plus ends and those at the kinetochore-ward edge of the ARB are newly created minus ends, because spindle microtubules are oriented with their plus ends towards the equator (McIntosh and Euteneuer, 1984).

Kinetochore microtubule dynamics in vivo can be studied by observing positions of ARBs on chromosomal spindle fibres. The two edges of an ARB in crane-fly spermatocytes were reported to move poleward with the same velocity (Forer, 1965, 1966), suggesting that tubulin subunits were being incorporated into kinetochore microtubules at the kinetochore at the same rate that tubulin subunits were leaving the same microtubules at the pole, behaving as in the 'treadmilling' model of microtubule equilibrium (Margolis and Wilson, 1978; Margolis et al., 1978). However, later work revealed that the two ARB edges often behaved asymmetrically: the poleward edge frequently moved poleward faster than the kinetochore-ward edge, indicating that ARB movement could not be due solely to 'treadmilling' (Wilson and Forer, 1989).

Chromosomal fibre ARBs by now have been studied in several animal cells. In general, the poleward edges of ARBs move poleward, but the rates of movement are different in different systems: in newt fibroblast and PtK cells the poleward ARB edge moves at 20 μm/min (Spurck et al., 1990), in grasshopper spermatocytes it moves at 5 μm/min (Gordon, 1980), and in crane-fly spermatocytes it moves at 1-2 μm/min (Wilson and Forer, 1988, 1989). Poleward ARB edges also move poleward in diatom spindles (Leslie and Pickett-Heaps, 1983, 1984), although these are not on chromosomal spindle fibres. In general, the kinetochore-ward edges of ARBs are stable, but they behave dif-

ferently in different cells: in newt fibroblasts and PtK cells they do not move (Spurck et al., 1990), in grasshopper spermatocytes they sometimes move poleward and sometimes do not (Gordon, 1980), and in crane-fly spermatocytes they always move poleward immediately after they are formed (Wilson and Forer, 1989). The presence of two ARBs on the same chromosomal spindle fibre alters ARB behaviour (Wilson and Forer, 1989): the region between the two ARBs becomes unstable, and the poleward movement of the kinetochore-ward ARB edge closest to the chromosome is usually delayed by the presence of a second ARB.

Several general conclusions can be made from the above summary of irradiations of animal cell chromosomal spindle fibres: (1) severed kinetochore microtubules attached to spindle poles are relatively unstable: they depolymerize at rates specific for each cell type; (2) severed kinetochore microtubules attached to kinetochores are stable and can elongate; and (3) severed kinetochore microtubules with both ends free are unstable and rapidly depolymerize.

ARBs in plant cells have been less extensively studied than in animal cells. In one study, both the irradiated region and the portion of the spindle fibre poleward from the irradiated region disappeared after one spindle fibre was irradiated in one metaphase *Haemanthus* endosperm cell; then, in 'a few minutes', the birefringence in the irradiated region 'returned' (Inoué, 1964). In two other studies with *Haemanthus* endosperm cells, electron microscopy showed that microtubule numbers were reduced in the irradiated and adjacent regions (Bajer and Molé-Bajer, 1970; Bajer, 1972).

We have studied ARB behaviour on chromosomal spindle fibres in *Haemanthus* endosperm cells, to compare it with ARB behaviour in animal cells. Our results suggest that spindle poles and kinetochores may influence the behaviour of kinetochore microtubules.

MATERIALS AND METHODS

Haemanthus katherinae were grown in greenhouses in Eugene, Oregon (USA), and either plants or fruits were brought (or sent) to Toronto, Ontario (Canada), where the experiments were performed. To prepare cells for irradiation and viewing, we selected fruits of the right age and removed the green outer layers from the fruits. We then prepared agar-coated coverslips. We spread molten phytoagar (at 0.5% concentration in 3.5% glucose; Bajer and Molé-Bajer (1986)) onto a 12 mm diameter glass coverslip. We then cut off one end of the naked fruit, removed the endosperm liquid by insertion of a Pasteur pipette, and placed endosperm liquid on the surface of a clean, 0.35×27×25 mm quartz coverslip (ESCO, Oak Ridges, NJ, USA) that was attached over the hole in an aluminum 'slide'. We placed an agar-coated coverslip on top of the endosperm liquid, agar side down, blotted off excess endosperm fluid with bibulous paper, and then covered the preparation with No. 27 halocarbon oil (Halocarbon Products Corp., Hackensack NJ, USA) to prevent evaporation. Cells in these preparations were flattened to different degrees. In extremely flattened cells the chromosomes were well separated, the individual chromosomal spindle fibres were distinct, and the polar regions were very broad, and thus the cells looked like the cells illustrated in other studies of *Haemanthus* endosperm (e.g. Figs 1,2). In rounder cells, the chromosomes were closer together, and the individual chromosomal spindle fibres were less distinct and were more focussed toward the polar regions (e.g. Fig. 3).

We viewed the preparations quartz side down using an inverted microscope (Wilson and Forer, 1987). Cells lived and divided under these conditions for several hours. After irradiating a cell, we often continued observations until the cell in question entered anaphase.

Irradiations utilized ultraviolet microbeam techniques described previously (Wilson and Forer, 1987; Czaban and Forer, 1991), using a 150 W Xenon lamp for irradiations with light of wavelength 260 nm, and a 100 W mercury-arc lamp for irradiations with light of wavelengths 270, 280 or 290 nm (see Forer, 1991), both lamps being purchased from UVP (San Gabriel, CA, USA). The monochromator was either from PTR Optics (Waltham, MA, USA) or from Oriel Corporation (Stamford, CT, USA). For all irradiations, the exit and entrance slits of the monochromator were of equal widths, corresponding to a 3.6 nm half-band-width of the transmitted light. The ultraviolet light was focussed to a circle of 1.3 μm in diameter in the cell.

Cells were studied using polarization microscopy, and were videotaped in real time before, during and after the irradiation, as described previously (Wilson and Forer, 1987). In irradiating cells, we (a) determined the position of the focussed ultraviolet beam using uranyl acetate (Czaban and Forer, 1991), (b) marked the position on the video monitor and closed the shutter, (c) found a cell, (d) aligned a spindle fibre to the mark on the video monitor, (e) opened the shutter to allow ultraviolet light to enter the microscope, and then (f) closed the shutter again once the ARB formed. We recorded the video images for varying time periods after the irradiation.

For plotting positions of ARB edges at different times, we played back the videotape, 'grabbed' the images we wanted using a 'frame grabber' (Coreco Inc., St. Laurent, Quebec, Canada), and marked with a cursor the positions of the points to be measured. A computer recorded the pixel positions and subsequently we used a computer programme to obtain distances (in the cell) between different pixel positions (see Wilson and Forer, 1989); we plotted distances versus time using a commercially available programme (SlideWrite Plus, Advanced Graphics Software, Inc., Sunnyvale, CA, USA), and used that same programme to calculate the least-mean-squares lines and the 'residuals' from those lines. Sometimes we enhanced the images via the computer, as an aid to identifying the edges of the ARB, or in determining what exactly happened in a particular cell. We prepared illustrations from the videotaped images using a P40U Mitsubishi video copier.

RESULTS

We produced ARBs on *Haemanthus* endosperm spindle fibres by irradiations with all wavelengths used. We successfully irradiated spindle fibres in 34 *Haemanthus* endosperm metaphase cells; in each cell the birefringence in the irradiated area was reduced, creating a discontinuity in the birefringence along the chromosomal spindle fibre (Fig. 1).

Chromosomal spindle fibres on the poleward sides of the ARBs were unstable and rapidly disappeared (e.g. Figs 1,2,3). The poleward fibres disappeared during or shortly after the irradiation, often too fast to be monitored during playback of the videotapes, but sometimes slowly enough to monitor. As seen in graphs of distance versus time, the chromosomal spindle fibre of the poleward side of the ARB shortened as if it was depolymerizing at the poleward edge (Fig. 4).

We were able to determine clearly the positions of the

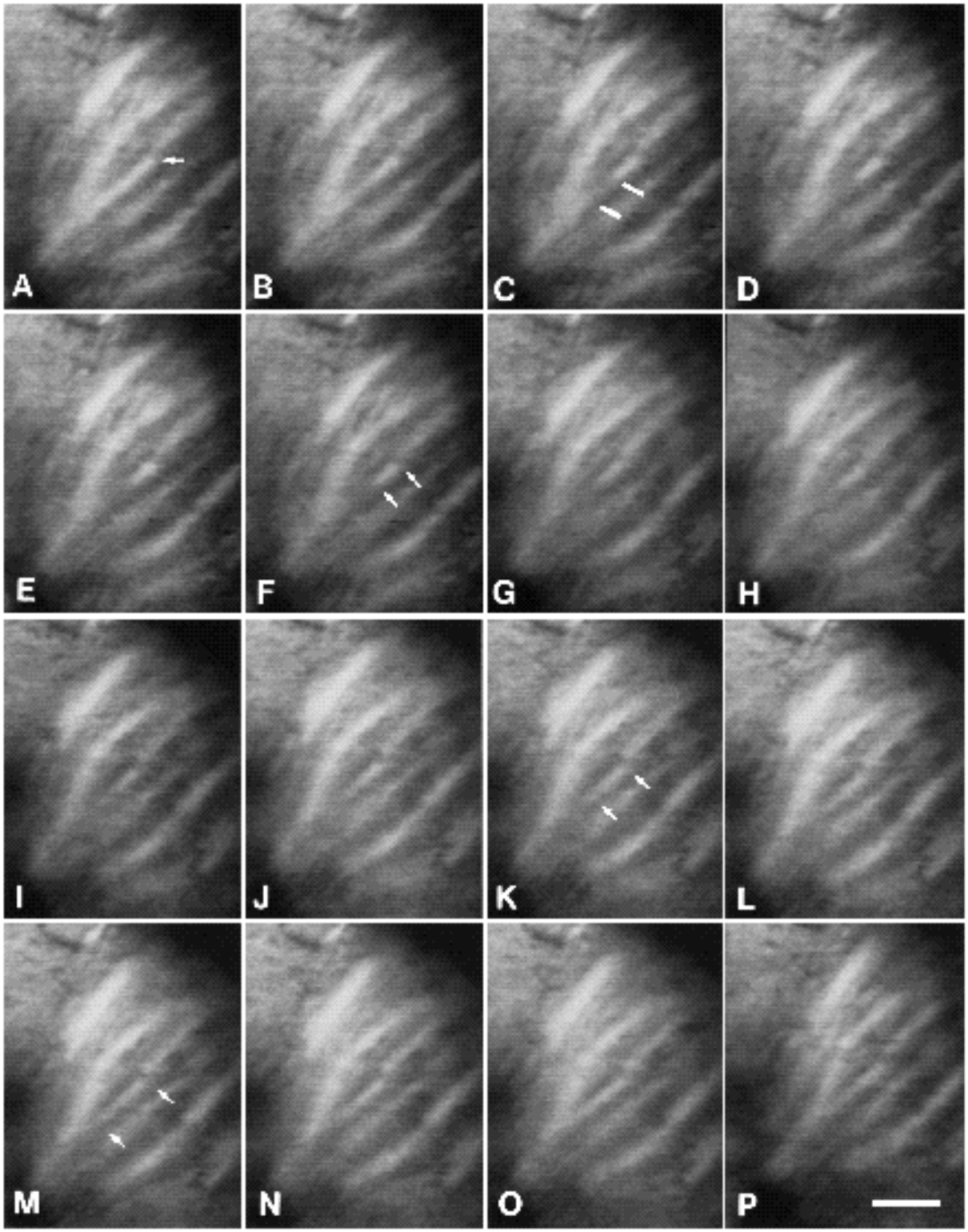

Fig. 1. ARB behaviour in a metaphase *Haemanthus* endosperm cell that was irradiated with 260 nm wavelength light and viewed with polarization microscopy. (A) was taped at 14:39:20, and corresponds to 0:00 (in minutes:seconds): (B) 0:20; (C) 0:23; (D) 0:31; (E) 0:50; (F) 1:00; (G) 2:06; (H) 2:40; (I) 5:23; (J) 6:40; (K) 7:10; (L) 7:50; (M) 9:40; (N) 10:40; (O) 11:50; (P) 13:50. (A) The cell before irradiation. The arrow indicates the chromosome associated with the irradiated fibre (to the lower pole). (B-C) The ARB is forming. The poleward and kinetochore-ward edges of the ARB are indicated by the small bars (in C). (D-E) After ARB formation, the chromosomal spindle fibre on the poleward side of the ARB rapidly disappeared. (F-P) The chromosomal spindle fibre on the kinetochore side of the ARB elongated, as indicated by the small arrows (F,K,M) at the kinetochore and at the end of the fibre. Bar, 10 μm.

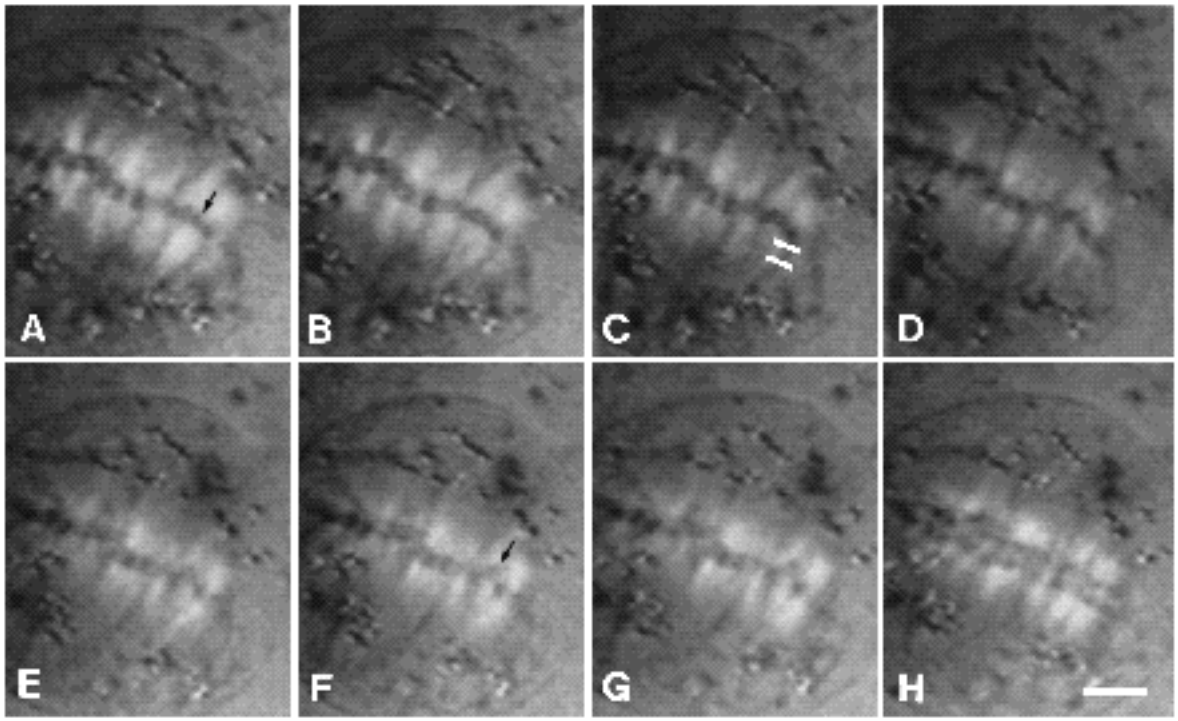

Fig. 2. ARB behaviour in a metaphase *Haemanthus* endosperm cell that was irradiated with 280 nm wavelength light and viewed with polarization microscopy. (A) was taped at 10:34:04, and corresponds to 0:00 (in minutes:seconds): (B) 0:37; (C) 1:14; (D) 3:09; (E) 6:03; (F) 7:04; (G) 10:02; (H) 18:17. (A) The cell before irradiation. The arrow indicates the chromosome associated with the irradiated fibre (to the lower pole). (B,C) Immediately after irradiation and ARB formation (2 small bars in C), the chromosomal spindle fibre on the poleward side of the ARB rapidly disappeared; the associated chromosome moved toward the non-irradiated half-spindle. (D-G) The chromosomal spindle fibre on the kinetochore side of the ARB started to elongate while the associated chromosome (arrow in F) moved further away from the equator (compare C with D-G). (H) The cell entered anaphase. Bar, 10 μm.

poleward edge of the ARB in only five cells because the poleward edge of the ARB diminished in birefringence towards the pole and was obscured by granules and chromosomal arms present in the polar region. In these five cells the average velocity (± standard deviation) with which the poleward ARB edges moved away from the kinetochores was 48 ± 26 μm/minute, as determined from the best-fit lines. In repeat measurements of the same cells, however, the velocities varied by factors of 3 to 5: with only 5 to 10 points in a time period of 5 to 10 seconds, differences in a few of the points had large effects on the slopes of the lines. Therefore, the velocities of the poleward ARB edges should be taken as an indication of order of magnitude of velocities, rather than hard and fast values.

Chromosomal fibres on the kinetochore sides of the ARBs were stable, and in all cells the fibres elongated after the ARB formed (Figs 1,2,3). As seen in graphs of distance versus time, the distance between the ARB edge and kinetochore did not begin to increase until 60 to 100 seconds after ARB formation (e.g. Fig. 4), as judged by eye. After the initial 'lag period', the distance between the kinetochore and the kinetochore-ward ARB edge increased linearly with time (Fig. 4). To test whether the 'lag period' that we judged 'by eye' indeed represented systemmatic deviation from linearity, we plotted the 'residuals' for the best-fit line through all the points. The 'residuals' - i.e. the distances of the points from the best-fit lines - were consistently positive or negative with respect to the line in the period we judged to be the 'lag period' (Fig. 4B (inset)), confirming that there was systematic deviation from linearity, and confirming our assessment that there were lag periods. To determine elongation rates, we first estimated by eye the length of the 'lag periods', and then we used the points after the lag periods to estimate the best-fit lines. For 10 cells, the average velocity (± standard deviation) with which the kinetochore-ward ARB edge moved away from the kinetochores, after an average 'lag period' of about 84 seconds, was 0.65 ± 0.28 μm per minute (with R-values for the best-fit lines ranging from 0.75 to 0.95), as summarized in Table 1.

There was one exception to these conclusions. In one cell, the chromosomal spindle fibres on the pole side of the ARB did not rapidly disappear. Rather, both edges of the ARB moved poleward at the same rate (Fig. 5).

The angle of the chromosomal spindle fibre on the kinetochore side of an ARB often changed after the irradiation. Before irradiation each chromosomal spindle fibre was oriented towards the pole; after formation of an ARB the fibre changed its angle in many cells (e.g. Fig. 3). Sometimes the fibre returned to its original orientation during elongation (e.g. Fig. 3), but sometimes the fibre remained at a different angle.

The chromosome associated with the irradiated chromosomal spindle fibre often moved off the equator after the

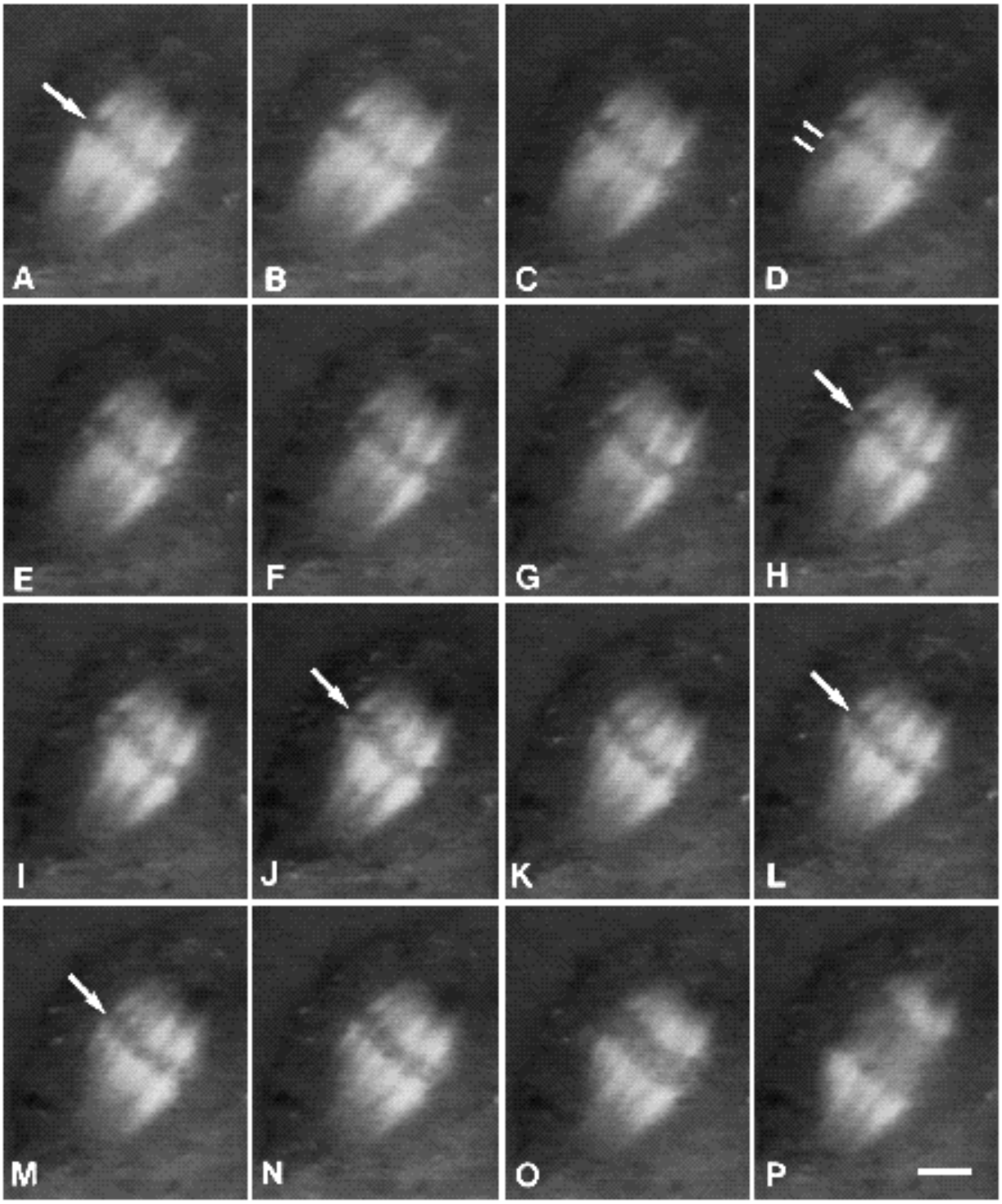

Fig. 3. ARB behaviour in a metaphase *Haemanthus* endosperm cell that was irradiated with 280 nm wavelength light and viewed with polarization microscopy. (A) was taped at 13:47:55, and corresponds to 0:00 (in minutes:seconds): (B) 0:10; (C) 0:15; (D) 0:25; (E) 0:35; (F) 1:04; (G) 1:45; (H) 3:05; (I) 6:35; (J) 9:05; (K) 11:23; (L) 13:47; (M) 16:26; (N) 18:25; (O) 20:53; (P) 25:48. (A) The cell before irradiation. The arrow indicates the chromosome associated with the irradiated fibre (to the lower pole). (B-F) An ARB was formed at the site of irradiation (2 small bars in D); the chromosomal spindle fibre on the poleward side of the ARB rapidly disappeared. (G,H) The chromosomal spindle fibre on the kinetochore side of the ARB started to elongate, minutes after ARB formation, at an angle to the spindle. As the fibre elongated, the associated chromosome (arrow in H) moved off the equator toward the non-irradiated half-spindle. (I-M) The fibre became re-oriented along the spindle axis (its original position) as it elongated; the associated chromosome (arrow in J,L,M) moved back towards the equator. (N) Anaphase started before the elongating fibre reached its initial length and before the associated chromosome reached the equator. (O,P) Anaphase continues. Bar, 10 μm.

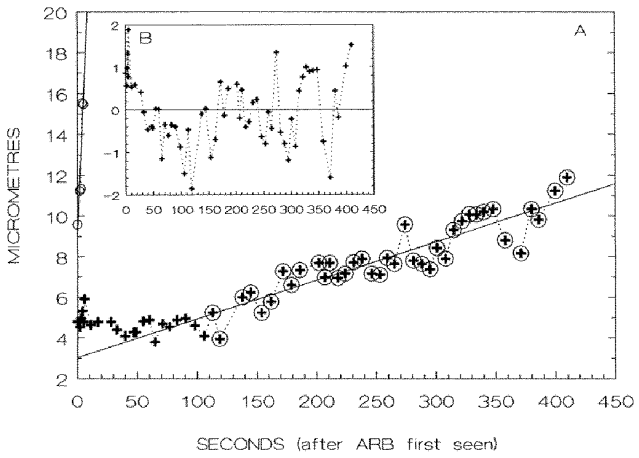

Fig. 4. Distance versus time graphs for ARB edge motion in a cell in which one chromosomal spindle fibre was irradiated with light of wavelength 290 nm. (A) The positions of the poleward edge (○) and the kinetochore-ward edge (+) of the ARB are plotted with respect to the kinetochore associated with the irradiated chromosomal spindle fibre. Time 0 is 18:52:10, the time at which the ARB was first seen. The 'best-fit' line for the kinetochore-ward edge positions is drawn only through the circled points, those after the initial 100 seconds. (B) The 'residuals' for the best-fit line through ALL the points in A. The residuals (i.e. the difference in the Y-direction between the positions of the actual points and the positions of the points predicted from the best-fit line) are positive from 0 seconds to 30 seconds, and primarily negative from 30 seconds to about 170 seconds, indicating that the points deviate systematically from the best-fit line.

irradiation (e.g. Figs 2,3). These chromosomes remained on the equator during the initial 'lag period', before the fibres started elongating, and then at about the time the fibre started to elongate they moved off the equator, generally for a distance of 2 to 4 μm, towards the non-irradiated half-spindle (see Figs 2,3). The chromosomes remained off the equator as the irradiated spindle fibre elongated, and often moved back towards the equator as the spindle fibres approached their initial lengths. Anaphase sometimes started before the chromosomal spindle fibre on the kinetochore side of an ARB elongated to its initial length; in such cells the chromosome moved poleward with normal velocity even though the irradiated fibre was shorter than the others (e.g. Figs 2,3).

Table 1. Summary of results from ultraviolet microbeam irradiations in *Haemanthus* endosperm

	Average value±standard deviation (*n*=numbers of cells)
Poleward side of ARB:	
Velocity of poleward motion	48±26 μm/min
Kinetochore side of ARB:	
Lag period before poleward motion starts	84±27 s (*n*=10)
Velocity of poleward motion AFTER the lag period	0.65±0.28 μm/min (*n*=11)*

*n=11 includes the cell in which both sides of the ARB move with the same speed, which is not included in those cells for which n=10.

DISCUSSION

In our experiments, ultraviolet microbeam irradiation produced ARBs on chromosomal spindle fibres in *Haemanthus* endosperm cells. In earlier reports, the birefringence poleward from the irradiated site disappeared simultaneously with that at the irradiated site (Inoué, 1964) and microtubule numbers were lower in both irradiated and adjacent regions (Bajer and Molé-Bajer, 1970; Bajer, 1972), suggesting that ARBs did not form. By observing the cells while the irradiation took place we saw that ARBs indeed were formed. In all 34 cells that we studied, the birefringent chromosomal spindle fibres poleward from the ARBs disappeared rapidly and the 'return' of birefringence of the irradiated fibre always occurred by 'elongation' of the remaining kinetochore fibre: we never saw the return of birefringence without elongation, which was suggested by Inoué (1964).

Thus, after irradiation of *Haemanthus* endosperm chromosomal spindle fibres, ARBs form, the kinetochore microtubules poleward from the ARBs rapidly depolymerize, and the kinetochore microtubules kinetochore-ward from the ARB elongate. Our results are compared in Table 2 with those from ultraviolet microbeam irradiation of other spindles in vivo and of microtubules in vitro. In the latter experiments, MAP-free brain tubulin was polymerized onto flagellar axonemes and, under conditions which favoured microtubule polymerization, the newly polymerized microtubules were severed by ultraviolet microbeam irradiation: newly created plus ends depolymerized very rapidly whereas newly created minus ends elongated, with no change in rate (Walker et al., 1989). Several conclusions can be reached from Table 2. One concerns the issue of where the kinetochore microtubules polewards from the ARB depolymerize. The spindle fibre appears to depolymerize from the plus end, the end closest to the kinetochore, but it is possible that depolymerization occurs at the pole and that the kinetochore microtubules are pulled poleward during depolymerization. Since depolymerization occurs from the severed plus ends of microtubules irradiated in vitro, it seems likely that depolymerization occurs from the severed plus ends of kinetochores in *Haemanthus* spindles in vivo.

Another conclusion is that newt fibroblast and PtK cells seem unique in that the remnant kinetochore microtubules attached to the kinetochore do not elongate: in all other cells the remnant kinetochore microtubules elongate - or, at least sometimes elongate - after irradiation. In newt fibroblast and PtK cells, within a few minutes after an ARB is produced the pole moves towards the equator to 'fill in' the ARB (Spurck et al., 1990; Snyder et al., 1991); it may be that the remnant kinetochore microtubules could grow, but that any growth is obscured by this 'filling in'.

Another conclusion is that crane-fly spermatocytes seem unique in the relative stability of the kinetochore microtubules poleward from the irradiated site: in all other cells those kinetochore microtubules shorten at much faster rates. One explanation for this could be that since MAPs stabilize microtubules in vitro against rapid depolymerization events (Horio and Hotani, 1986; Hotani and Horio, 1988), it may be that kinetochore microtubules in crane-fly sper-

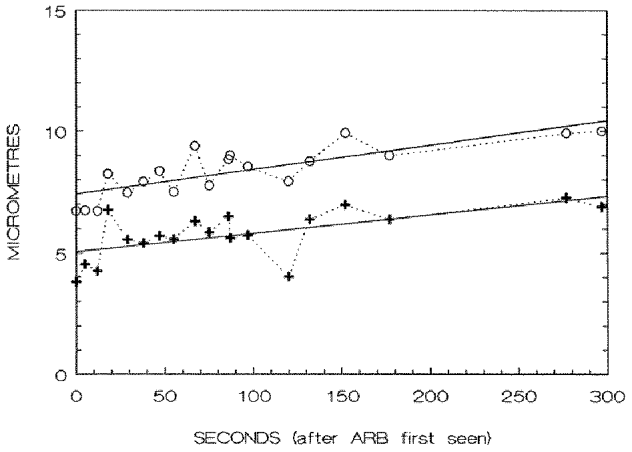

Fig. 5. Distance versus time graph for ARB edge motion in the exceptional cell described in the text. The positions of the poleward edge (○) and the kinetochore-ward edge (+) of the ARB are plotted with respect to the kinetochore associated with the irradiated chromosomal spindle fibre. The 'best-fit' lines are drawn through all points. Both edges of the ARB move away from the kinetochore at almost constant rates.

matocytes are associated with MAPs whereas kinetochore microtubules in the other cells are not. Results from double irradiation experiments of crane-fly spermatocytes (Wilson and Forer, 1989) suggest another possibility, that kinetochore microtubule stability poleward from the ARB in crane-fly spermatocytes is influenced by the spindle poles. When two ARBs were formed on a single chromosomal spindle fibre, the kinetochore microtubules poleward from the ARB closer to the spindle pole were connected to the pole and were stable, while the kinetochore microtubules poleward from the ARB closer to the kinetochore were severed from the pole (by the other ARB) and were unstable and rapidly shortened. This suggests that the crane-fly spermatocyte spindle pole stabilizes the attached kinetochore microtubules, with a necessary corollary being that the poles in the other cells do not.

The same experiments are relevant to another issue, the time lag between severing of the kinetochore microtubules and growth of the microtubules on the kinetochore side of the ARB in *Haemanthus* endosperm compared to the absence of a time lag in crane-fly spermatocytes. When there is only one ARB on a single spindle fibre in crane-fly spermatocytes, the remnant kinetochore microtubules attached to the kinetochore immediately grow poleward, but when there are two ARBs, the remnant microtubules attached to the kinetochore do not start growing poleward until after a time lag (Wilson and Forer, 1989). Since free minus ends of kinetochore microtubules should behave the same whether there are one or two ARBs per fibre, the difference would seem to lie elsewhere, and since in the double irradiation experiments the remnant microtubules attached to the kinetochore were not connected to the pole, it seems likely that the kinetochore determines the growth rate of the attached kinetochore microtubules, probably by controlling polymerization at the kinetochore end. It seems to us, therefore, that the differences in ARB behaviour in different types of cells (or in the same cell under different conditions) are most easily explained as being due to kinetochores and poles influencing the attached microtubules. This suggests to us that kinetochores and poles not only 'cap' microtubules, but, at least in part, determine the behaviour of the attached microtubules.

A final conclusion from the comparisons in Table 2 would seem to be that results from one cell type do not necessarily pertain to others, and, therefore, that we need to study more cell types before we can reach general conclusions about the behaviour of free microtubule ends in spindles in vivo.

In our experiments, the kinetochore microtubules on the kinetochore side of the ARB did not always elongate in the direction of the original chromosomal fibre. We assume that this is because the irradiation caused the disappearance of non-kinetochore microtubules, and that without non-kinetochore microtubules the paths of elongation were free to be in any direction. In support of this conjecture we note that the growing chromosomal spindle fibres often changed their growth directions when they came near enough to other fibres to be able to be affected by them, and we note that after colcemid treatment of crane-fly spermatocytes chromosomal spindle fibres often grew back at altered angles, independent of each other and of the original pole-to-pole direction (Czaban and Forer, 1985).

In our experiments, the chromosome associated with an

Table 2. Behaviour of microtubules after being severed by ultraviolet microbeam irradiation

	Rate of kMT elongation at k-end of ARB in μm/min	Rate of kMT shortening at p-end of ARB in μm/min	Delay before kMTs elongate in s
Haemanthus endosperm (from Table 1)	0.65±0.28 (*n*=11)	48±26 (*n*=5)	84±27 (*n*=10)
Grasshopper spermatocytes (from Gordon, 1980)	~0.5 (*n*=3)	~5 (*n*=8)	–
Newt fibroblast and PtK cells (from Spurck et al., 1990)	0 (*n*=83)	20±0.6 (*n*=83)	–
Crane-fly spermatocytes* (from Wilson and Forer, 1989)	0.76±0.31 (*n*=17)	1.04±0.48 (*n*=17)	0 (*n*=17)
MAP-free MTs in vitro (from Walker et al., 1989)	0.40±0.03 (*n*=28)	20±1.5 (*n*=15)	0

*Data from experiments with single ARBs.
Abbreviations: MTs, microtubules; kMT, kinetochore microtubules; k-end, kinetochore end of an ARB; p-end, pole end of an ARB.

irradiated fibre often moved off the equator. Since force produced by a chromosomal spindle fibre is proportional to the fibre's length (Hays et al., 1982), one would expect the chromosome to move immediately toward the non-irradiated half-spindle, since after irradiation that side has the longer chromosomal spindle fibre. The chromosomes in *Haemanthus* endosperm did indeed move towards the non-irradiated half-spindle, but, contrary to expectation, the movement of the chromosome off the equator did not occur immediately after the ARB was formed, only some minutes later, and often after the irradiated spindle fibre had already begun to elongate. One would also expect the chromosome to move back to the equator as the irradiated fibre elongates, and to reach the equator only when the chromosomal spindle fibres to the two sides are of equal length. Contrary to this expectation, too, in *Haemanthus* endosperm the chromosomes often continued to move away from the equator while the irradiated fibres elongated, and the chromosomes returned to the equator before the irradiated fibres reached their original lengths. We do not know why these behaviours differ from expectation.

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