ACTION SPECTRUM FOR CHANGES IN SPINDLE FIBRE BIREFRINGENCE AFTER ULTRAVIOLET MICROBEAM IRRADIATIONS OF SINGLE CHROMOSOMAL SPINDLE FIBRES IN CRANE-FLY SPERMATOCYTES

PEGGY J. SILLERS AND ARTHUR FORER
Biology Department, York University, Downsview, Ontario M3J 1P3, Canada

SUMMARY

Single chromosomal spindle fibres in Nephrotoma suturalis (crane-fly) spermatocytes in metaphase and anaphase were irradiated with monochromatic ultraviolet light focussed to a 2 μm spot. In cells in both metaphase and anaphase either the birefringence of the irradiated spindle fibre was altered in the irradiated region, or there was no change, depending on the dose and wavelength of ultraviolet light used for the irradiation. When there was an area of reduced birefringence (ARB), it moved poleward regardless of whether the associated chromosome moved poleward. When cells were irradiated in early metaphase they remained in metaphase until the ARB reached the pole. In some cells irradiated in late metaphase the chromosomes began anaphase before the ARB reached the pole; in many such cells anaphase was abnormal in that all six half-bivalents separated at the start of anaphase but none moved polewards.

In all cases the ARB moved poleward at the same speed as subsequent chromosome movement; that is, at about 0.8 μm/min.

In cells irradiated in anaphase, spindle fibre birefringence was reduced independently of blockage of chromosome movement. Because birefringence and movement were altered independently there were four classes of results: (1) in some cases there was no effect on the movement of the chromosome associated with the irradiated spindle fibre and no effect on the birefringence of the irradiated spindle fibre. (2) In some cases, primarily with 260 nm wavelength light, there was no effect on the movement of the chromosome associated with the irradiated spindle fibre and there was an effect on the birefringence of the irradiated spindle fibre. (3) In some cases, primarily with 290 nm wavelength light, there was an effect on the movement of the chromosome associated with the irradiated spindle fibre and there was an effect on the birefringence of the irradiated spindle fibre. (4) In some cases, primarily with 270 nm and 280 nm wavelength light, there was an effect on the movement of the chromosome associated with the irradiated spindle fibre and there was an effect on the birefringence of the irradiated spindle fibre.

The action spectrum for reducing spindle fibre birefringence in crane-fly spermatocytes had two peaks, one at 260 nm and the other, less sensitive, at 280 nm. For irradiations at 270 nm, 280 nm and 290 nm, five to fifty times more energy was needed to reduce spindle fibre birefringence than to stop chromosome movement, but for irradiations at 260 nm five times less energy was needed to reduce spindle fibre birefringence than to stop chromosome movement. The action spectrum for reducing spindle fibre birefringence is quite different from that for stopping chromosome movement.

INTRODUCTION

Action spectra are measures of the efficacy of light of different wavelengths in producing a measured effect (an 'action'). To obtain an action spectrum one irradiates
with graded doses of any one wavelength, then finds a dose for a 50% effect, and then compares the 50% doses of the different wavelengths to see which wavelengths are most effective in giving the observed response. The curve of 50% dose needed for the action versus the wavelength is called the ‘action spectrum’ for that particular effect.

Action spectra are useful for several reasons. One is that an action spectrum may indicate if more than one chromophore is involved in the observed effects: different peaks in the action spectrum correspond to the different absorption peaks of the different chromophores. (The term 'chromophore' refers to that part of a molecule that absorbs light of the particular wavelength in question — for example, absorption of light of wavelength 290 nm by tryptophan residues in a protein.) A second reason is that an action spectrum may give chemical details of the components involved; these are obtained by comparing the action spectrum to absorption spectra of suspected components, or to action spectra of purified components.

There are some ambiguities inherent in interpreting action spectra at the molecular level (e.g., see Wetlaufer, 1962). For example, an action spectrum with two peaks indicates that there are at least two different chromophores involved, but these two might be part of a single molecular component, or they might each be part of different molecular components. Further, single peaks in action spectra do not necessarily represent only one chromophore, because different chromophores can have similar absorption spectra. Even if a single peak in an action spectrum is due to one single chromophore, this could still represent several different molecular components, all of which have the same chromophore; for example, several proteins that all have tryptophan residues near active sites. Thus one can use an action spectrum to specify a minimum number of chromophores involved, but this number is not necessarily the same as the number of molecular components involved.

In this paper we describe an action spectrum for reducing the birefringence of portions of individual spindle fibres that were irradiated with monochromatic ultraviolet light. We have done these experiments for several reasons. One is to test which components are responsible for the birefringence of the spindle fibres. Several workers (e.g., see Inoué, 1981; Bajer & Mole-Bajer, 1981) believe that microtubules are the sole contributors to spindle fibre birefringence. Others (e.g., see Forer, 1976, 1978) have argued that not only do microtubules contribute to the birefringence of spindle fibres but several other components such as actin, myosin and dynein may be contributing as well. By determining an action spectrum for changes in spindle fibre birefringence we then can study the structural changes seen after an irradiation, for example, using electron microscopy or staining with fluorescent antibodies. Such experiments can determine directly what the birefringence in the spindle fibre is due to.

Another reason for studying the action spectrum for changes in spindle fibre birefringence is to compare it with the previously determined action spectrum for reversibly blocking chromosome movement (Sillers & Forer, 1981c). When individual spindle fibres of cells in anaphase were irradiated with an effective dose, the chromosome associated with the irradiated spindle fibre temporarily stopped moving,
for 2–15 min, whereas other chromosomes moving to the same pole moved normally. The action spectrum for blocking chromosome movement had two peaks (at wavelengths 270 nm and 290 nm) and exactly matched that for blocking myofibril contraction, but was quite different from that for blocking ciliary beating (Sillers & Forer, 1981c). The action spectrum for changes in spindle fibre birefringence presented in this paper does not match that for blocking chromosome movement.

A final reason for studying the action spectrum for reducing spindle fibre birefringence is to clarify previous results using heterochromatic ultraviolet light (Forer, 1966); those results were quite unpredictable, the interpretations were very complicated, and McIntosh (1979) has suggested that the experiments should be re-evaluated. The action spectra presented herein greatly clarify the previous results.

**MATERIALS AND METHODS**

We irradiated single autosomal spindle fibres in crane-fly spermatocytes using procedures that have been described (Sillers & Forer, 1981a), with minor modifications. One modification was the species of crane-fly: previously we used *Nephrotoma ferruginea* (Fabricius), but the considerable continuous fibre birefringence in spermatocytes often obscures the chromosomal spindle fibres. The experiments reported here used *Nephrotoma suturalis* (Loew) because there is much less continuous birefringence in these spermatocytes than in those of *N. ferruginea*. The ultraviolet microscope was modified for polarization microscopy, as follows. The light source for viewing was a 200 W mercury arc lamp (Illumination Industries Inc.) housed in a Reichert lamp housing; prior to entering the condenser the light for viewing passed through an HN-22 polaroid polarizer and a homemade mica compensator (cleaved mica mounted between two coverslips). The mica compensator was calibrated against a commercial mica compensator, and retardation was λ/16. The condenser was a long-working-distance Nikon condenser, selected to be strain-free and used at a numerical aperture (NA) of 0.5. The objective was a Reichert glycerol immersion lens, 60x (NA 0.95) also selected to be strain-free. The Reichert lens was switched to the ultraviolet (u.v.)-transmitting Zeiss 100X phase-contrast Ultrafluar glycerol immersion lens (NA 0.85) by swinging a turret that had been modified so that both lenses were centred with respect to each other and approximately parfocal with each other. At the same time the diaphragm on the condenser was removed and replaced with a 100X phase ring. The Zeiss Ultrafluar lens was used for viewing and irradiating cells as described previously (Sillers & Forer, 1981a). For most irradiations reported here the ultraviolet light was focussed to a spot in the cell of 2-1 μm in diameter. Immediately following an irradiation the procedure was reversed and the cell was observed using polarizing microscopy.

We selected times of irradiations and total doses as described previously (Sillers & Forer, 1981a). The lengths of times for irradiations at each wavelength were in the following ranges: 260 nm, 20–375 s; 270 nm, 5–40 s; 280 nm, 15–90 s and 290 nm, 3–68 s. Since very long irradiation times were sometimes required to obtain the desired doses, especially using 260 nm wavelength light, we replaced the previously used Schoeffel GM-100 monochromator with an Oriel 7270 monochromator; the irradiation times for 260 nm were reduced fourfold by the new monochromator. We irradiated cells in both anaphase and metaphase; we often irradiated cells in metaphase when long irradiation times were needed to reduce spindle fibre birefringence. (If, for example, a cell was in anaphase and the movement of the chromosomes associated with the irradiated spindle fibre was unaffected by the irradiation, then the chromosome might move right into the irradiating spot.) Cells were photographed before, during, and after an irradiation using 16 mm film (Kodak Plus X Neg Film 7231) with compensator angles of 3° and fixed exposure times of about 6 s. Video images were also recorded using a Venus model DV2 (Venus Scientific Inc., New York) low light level video camera that had its automatic gain control off and a manual gain set at a specific level. The video image included date and time (using an RCA time-date generator) and was
recorded using a Javelin reel-to-reel video tape recorder (Absolute Security Ltd, Mississauga, Ont.). The video tape recorder was modified by Canadian Instrumentation and Research, Toronto, to remove its automatic gain control; all data were recorded with the automatic gain control inoperative. Images were generally recorded at the recording speed of '25 h'. The video images were viewed on a 17 inch Javelin TV monitor (Absolute Security Ltd). Video images were made for two reasons: (1) so that the effects of the irradiations could be seen immediately; thus we could quickly decide what range of doses to work in when irradiating cells; (2) so that we could quantify the birefringence of the individual chromosomal spindle fibres before and after an irradiation, using the system developed by Schaap & Forer (1981, and unpublished). Briefly, a digitizer is interfaced between the video tape recorder and a computer, and quantitative birefringence values are extracted along the length of the individual chromosomal spindle fibres.

For the illustrations we photographed the TV monitor using a Nikon F3 camera with a Micro-Nikkor 55 f/2-8 lens, with exposure times of 1/15 of a second and an f-stop of 5-6.

The curves for the action spectrum were determined as described previously (Sillers & Forer, 1981c), and were analysed statistically as described therein. Additional data for stopping chromosome movement were obtained (as described in that paper) and have been included here, that is, the stoppage of chromosome movement with irradiations using 260 nm wavelength light.

Several uncertainties in determining the energy incident on a spermatocyte at the time of an irradiation have been discussed previously (Sillers & Forer, 1981c); we also tested other possible sources of error. For 260 nm and 270 nm irradiations we used a xenon arc lamp. The spectral output of this lamp was measured by inserting the narrowest possible slit in the monochromator (half-band width of 2 nm) and measuring the intensities after the light left the monochromator: intensities were measured between 250 and 300 nm, at intervals of 2 nm, and, in agreement with the spectra given in the 1981—1982 Oriel catalogue, the output is linear, with no sharp peaks. Thus, when the monochromator was set to 260 nm (or to any other wavelength from 250 to 300 nm) there was no possibility that light from a sharp peak at a different wavelength would predominate and contribute more energy to the output than the selected wavelength itself. A mercury arc lamp was sometimes used for irradiations using 280 nm and 290 nm wavelength light. The mercury arc lamp has a line spectrum (peaks) superimposed upon a continuum; the lines are at 280 nm, 289 nm, 297 nm and 303 nm, and therefore with this lamp, too, there are no peaks at intermediate wavelengths that would contribute more energy to the output than the wavelength selected by the monochromator. These experiments also confirmed that the monochromator dial was calibrated properly, because all the lines were at the correct positions.

We also tested the spectral sensitivity of the P1100 silicon detector probe, to see if the manufacturer's calibration was accurate. The energy from a 6-5 A tungsten lamp source was measured at wavelengths ranging from 400 to 900 nm (half-band width of 10 nm) using both our P1100 probe and a Photodyne radiometer/photometer (model 88XL) with a Photodyne receiver (model 250). (We are grateful to Dr J. Miller of the Physics Department at York University, for letting us use his calibrated Photodyne equipment.) These calculated values were compared to the manufacturer's calibrated values by plotting the two measurements on a curve of energy versus wavelength. The difference between the two was less than 10% for wavelengths of 500—900 nm, and thus we are confident that our detector was calibrated accurately by the manufacturer. (The Photodyne detector is not sensitive to light of lower wavelengths, so we could not compare the two detectors using ultraviolet light of 250—300 nm wavelengths; we assume that since the calibrations were accurate using visible light, they will also be accurate using ultraviolet light.)

Crane-flies were reared as described elsewhere (Forer, 1982), and preparations of living spermatocytes were made as described previously (Sillers & Forer, 1981a, b).

RESULTS

General results of irradiations of autosomal spindle fibres with an ultraviolet microbeam

We irradiated single autosomal spindle fibres in crane-fly primary spermatocytes at metaphase or anaphase. We irradiated a total of 117 cells. Depending on the
Action spectrum for changes in spindle fibre birefringence

wavelength and dose used for the irradiation a localized area of reduced birefringence (an ARB) did or did not appear on the spindle fibre at the spot that was irradiated. That is to say, as judged by eye after the irradiation either the birefringence was normal or there was an ARB. A localized area of reduced birefringence (ARB) was noticeable immediately following an irradiation (i.e. within seconds); there was no case in which a spindle fibre appeared unaffected and then subsequently (i.e. several minutes later) showed the effect. Occasionally, when a very high dose was used to irradiate the spindle fibre, the total spindle birefringence (as judged by eye) decreased, whether an ARB was produced or not.

Metaphase cells were irradiated in most of the cases (93 out of 117). When an ARB was produced the localized spot moved poleward and disappeared at the pole (Fig. 1). In a total of 18 cells for which data are available the ARB moved at 0.76 ± 0.19 μm/min, which is slower than the speed of 1 μm/min of chromosome movements in these cells at 23°C (room temperature; Schaap & Forer, 1979). Anaphase speeds of chromosomes in cells that were irradiated in metaphase were not recorded, so we have no data on whether the velocity is normal in anaphase cells when an ARB is produced in metaphase.

When cells irradiated in metaphase entered anaphase shortly after the irradiation the poleward chromosome movement was sometimes abnormal, regardless of whether

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Fig. 1. A metaphase cell with an ARB (produced by u.v. light of 280 nm and a total incident energy of 0.08 ergs/μm² at the focussed spot). A. The cell before irradiation; subsequent photographs are after irradiation. In this cell the chromosomes separated during the irradiation but did not move poleward in anaphase until the spot reached the pole. A. 0 min (just prior to irradiation); B, 1 min after irradiation; C, 10 min after irradiation; D, 16 min after irradiation. ×1900.
Fig. 2. A metaphase cell before and after an irradiation (using u.v. light of 290 nm and a total incident energy of 0.1 ergs/μm² at the focussed spot) in which no ARB was produced. A, 0 min (i.e. just prior to irradiation); B, 1.5 min after irradiation; c, 3-5 min after irradiation. ×1900.

Fig. 3. A distance versus time graph of an anaphase cell with no stoppage of movement but with an ARB (produced using u.v. light of 260 nm, at a total incident energy of 0.2 ergs/μm² at the focussed spot). The ARB (□) and the chromosomes (▲, ●, ■) moved to the pole at approximately the same speed. Distance measurements for the chromosomes were obtained by measuring the interkinetochore distance between separating bivalents and the ARB was measured from the edge of the spot nearest the chromosome to the kinetochore of the associated chromosome. The black bar indicates when the irradiation took place.
Action spectrum for changes in spindle fibre birefringence

Fig. 4. Distance versus time graph depicting a 2.5 min stop of the half-bivalents associated with the irradiated spindle fibre (■). This spindle fibre also had an ARB produced after the irradiation (□). Although the bivalents associated with the irradiated spindle fibre stopped moving the ARB moved poleward immediately after the irradiation at approximately the same speed as subsequent chromosome movement. All other bivalents (▲, ●) moved normally after the irradiation. The distance measurements were made as described for Fig. 3. The wavelength used in this irradiation was 280 nm and the dose was approximately 0.4 ergs/µm². The black bar indicates when the irradiation took place.

An ARB was produced (e.g., see Fig. 2). Anaphase occurred shortly after irradiation in 22 cells. There was no ARB in 12 such cells, but anaphase was abnormal in six cells in that the chromosomes separated, but none moved poleward, even though only one or two chromosomal spindle fibres were irradiated. (In one cell this movement blockage was only temporary; we assume that it is temporary in all cells, as reported previously (Forer, 1966), but since we were concentrating on the action spectrum we did not follow these cells for very long.) There were ARBs in the other 10 cells, and anaphase was abnormal in four of these; the chromosomes separated but none of the six half-bivalents moved poleward, even though there were ARBs only on one or two of the chromosomal spindle fibres. (All four of these cells were irradiated with 280 nm wavelength light.) It is relevant to point out that in our previous work irradiations in anaphase blocked the movement of only the chromosomes associated with the irradiated fibre, never of all six chromosomes (Sillers & Forer, 1981b). The blocking of all six, then, is a result specific to metaphase.

Anaphase chromosome movement was sometimes normal when ARBs were

* The six cells with blocked movement were irradiated with the following wavelengths and doses of ultraviolet light: two cells at 260 nm at doses of 0.03 and 0.08 ergs/µm²; two cells at 270 nm at doses of 0.08 and 0.2 ergs/µm²; and two cells at 280 nm at doses of 0.2 and 0.4 ergs/µm².
Produced and cells entered anaphase shortly after the irradiation. This occurred in six out of 10 cells: five of these cells were irradiated with 260 nm wavelength light and one was irradiated with 270 nm wavelength light. These results are strikingly different from those reported earlier: Forer (1966) used a heterochromatic u.v. microbeam to irradiate crane-fly spermatocytes, and metaphase cells with an ARB that entered anaphase shortly after the irradiation never had normal anaphase movement (in 15 cells) when irradiated in metaphase (see fig. 9 of Forer, 1966). In our experiments anaphase movement was normal in six out of 10 cells. The two sets of results together strongly suggest that there are two different effects (changing birefringence, and blocking movement of all six half-bivalents), that are caused by different wavelengths.
Our data suggest that perhaps 280 nm wavelength light is the most effective wavelength for blocking movement in this way.

Anaphase cells were also irradiated (24 out of 117 cells). Depending on the dose and wavelength of u.v. light the movement of the chromosome associated with the irradiated spindle fibre (and its partner half-bivalent) was or was not blocked, and an ARB was or was not produced. In all, there were four different results with different combinations of the movement and birefringence effects, as follows.

(1) In three cells there was no stoppage of chromosome movement and no ARB was produced.

(2) In four cells there was no stoppage of chromosome movement but an ARB was produced. In these cells the distance between the kinetochore and the ARB remained approximately the same throughout anaphase; the ARB appeared to be moving at approximately the same speed as the chromosomes (Fig. 3). All four cells were irradiated with 260 nm wavelength light.
In 12 cells there was stoppage of chromosome movement but no ARB was produced. In these cases the birefringence of the irradiated spindle fibre appeared by eye to be unaltered, but the movement of the two partner half-bivalents associated with the irradiated spindle fibre stopped moving for 2–15 min as described previously (Sillers & Forer, 1981b, c). Of the 12 cells, seven were irradiated with 290 nm, two were irradiated with 280 nm and three were irradiated with 270 nm wavelength light.

In five cells there was stoppage of chromosome movement and an ARB was produced. In these cells the ARB moved poleward even though the chromosome associated with the irradiated spindle fibre stopped moving (Fig. 4). In the five cells the ARB movement was at 0·74 ± 0·2 μm/min. After the stopped chromosomes resumed movement, the distance between the kinetochore of the stopped chromosome and the ARB remained constant as both the chromosome and ARB moved poleward.

There were seven cells in which the dose used for the irradiation was so high that the entire spindle birefringence was reduced. This occurred at all wavelengths when high doses were used. No data from these cells are used in this paper, but it is relevant to point out that these cells were not dead; often the birefringence slowly returned, over several hours, to what seemed by eye to be normal levels, and the cells entered anaphase and completed meiosis normally.

We quantified the birefringence along various spindle fibres after cells were irradiated. Four cells were analysed in which there seemed by eye to be no change in spindle fibre birefringence. These included one cell irradiated with each wavelength; that is, at 260 nm, 270 nm, 280 nm and 290 nm. Three of these cells (those at 260, 270 and 280 nm) were in metaphase when irradiated and the other was in anaphase. In all cases no ARB was produced and the birefringence of the irradiated spindle fibre, the birefringence of the spindle fibre of the partner chromosome, and the birefringence of the control (not-irradiated) spindle fibre did not change at all (Fig. 5). Four cells were analysed in which it seemed by eye that an ARB was produced. These included two cells irradiated with 280 nm and two cells irradiated with 260 nm wavelength light. The irradiated area lost from 40–70 % of the birefringence originally present in that region of the spindle fibre (Fig. 6). Spindle fibres of partner chromosomes and of other unirradiated spindle fibres in the same cell were either unchanged in birefringence after an irradiation or the birefringence along the entire spindle fibre changed, by up to ± 10 %. Since changes of up to 25 % also occur in unirradiated cells (Forer, 1976; Schaap & Forer, 1981 and unpublished data), these changes were within normal limits and we conclude therefore that the irradiations had no effect on the unirradiated chromosomal spindle fibres.

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Fig. 6. Retardation versus the distance along the spindle fibre, in a metaphase cell in which an ARB was produced by a 4 μm diameter irradiating spot. A. The irradiated spindle fibre before irradiation; B, the partner spindle fibre before irradiation; C, the irradiated spindle fibre after irradiation using 280 nm wavelength light and a dose of 0·4 ergs/μm²; 40 % of the original birefringence is lost in the ARB area (between the arrows). D. The partner spindle fibre after irradiation; there is no change in birefringence. Other spindle fibres in the same cell, although not shown, did not change in birefringence after irradiation.
Action spectrum for changes in spindle fibre brightness.
Fig. 6c. For legend see p. 10.
Fig. 60. For legend see p. 10.
Fig. 7. Percentage of cells with an area of reduced chromosomal spindle fibre birefringence (ARB) after an irradiation versus the relative dose. The relative dose given under each graph differs for each wavelength because the sensitivity of the photocell recording the energy differs slightly for each wavelength. The EE50BR values were calculated from these graphs as shown. Each graph is for an individual wavelength as indicated at the top of each one.

There were no differences in the times that chromosomes stopped moving for cells irradiated in anaphase that had an ARB compared with cells irradiated in anaphase that did not have an ARB. The average stopping time for chromosomes in cells irradiated in anaphase that had an ARB after the irradiation was 2.9 ± 0.74 min (n = 5), whereas the average stopping time for chromosomes in cells irradiated in anaphase in which no ARB was produced after irradiation was 2.8 ± 0.83 min (n = 12). (The values are averages ± standard deviations.)
Action spectrum for changes in spindle fibre birefringence

We determined an action spectrum for producing ARBs. For each wavelength we plotted percentage of irradiated cells that showed an ARB versus dose; in each curve data from both metaphase and anaphase cells were used. From the curves (given in Fig. 7) we estimated the dose required for each wavelength to reduce spindle fibre birefringence 50% of the time. We did this both from the curves as drawn and from least-mean-squares analysis of semi-logarithmic plots of the same data (Sillers & Forer, 1981c); the results were very similar (Table 1). To obtain the action spectrum we plotted the EE50BR, in ergs/μm² versus the wavelength (Fig. 8). The action spectrum for producing ARBs has two distinct peaks, one at 260 nm and the other at 280 nm. Statistical tests, as described previously (Sillers & Forer, 1981c), verify that
both peaks indeed are different statistically from the EE_{50}BR values of the adjacent wavelengths. At the least sensitive wavelength used (290 nm) the EE_{50}BR is > 1.5 ergs/μm², more than 50 times more than the EE_{50}BR at the most sensitive wavelength (260 nm). Indeed, irradiations at 260 nm require five times less energy to produce an ARB than any of the other wavelengths. Since there are two peaks in the action spectrum there are at least two chromophores involved in the effect.

The action spectrum for changes in spindle fibre birefringence might be modified due to absorption by the chromosomal spindle fibre; that is, the absorption by other, non-affected spindle fibre components might, in theory, change the apparent action spectrum (discussed by Sillers & Forer, 1981c). To take this into account, we measured the absorption of a spindle fibre and corrected the apparent action spectrum by the appropriate absorption, as described previously (Sillers & Forer, 1981c). The modified action spectrum (given in Fig. 9) still has two peaks, at 260 nm and 280 nm, that are statistically significantly different from the adjacent points.

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Fig. 9. The action spectrum for production of an ARB on spindle fibres in crane-fly spermatocytes, obtained by plotting the EE_{50}BR values (in ergs/μm²) versus wavelength. There are two distinct peaks of sensitivity, at 260 nm and 280 nm.
Table 1. *Comparison of the EE\textsubscript{50BR} values calculated in two different ways*

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Estimation of EE\textsubscript{50BR} value from curves of percentage of cells with an ARB versus dose (ergs/\mu m\textsuperscript{2})</th>
<th>Calculation of EE\textsubscript{50BR} value from least-mean squares lines of percentage of cells with an ARB versus ln dose (ergs/\mu m\textsuperscript{2})</th>
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<tr>
<td>260</td>
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<td>270</td>
<td>0.4</td>
<td>0.38</td>
</tr>
<tr>
<td>280</td>
<td>0.3</td>
<td>0.25</td>
</tr>
<tr>
<td>290</td>
<td>Too high a dose is required</td>
<td>Too high a dose is required</td>
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</tbody>
</table>

The action spectrum for producing ARBs is quite different from that for blocking chromosome movement (Sillers & Forer, 1981c); the two complete sets of data are given in Fig. 10. We have extended the previous data for blocking chromosome movement at 260 nm and included these new data in Fig. 10. A composite graph showing the two action spectra, that for stopping movement and that for producing ARBs, is given in Fig. 11. It is clear from these data that one can produce an ARB.

![Fig. 9. The action spectrum for changes in spindle fibre birefringence modified by the absorption differences through individual spindle fibres at each wavelength, as described by Sillers & Forer (1981c). There are two distinct peaks of sensitivity, at 260 nm and 280 nm.](image-url)
without affecting movement, or one can alter movement without producing an ARB, or one can get both effects, just by choosing the appropriate wavelength and dose. Thus one can easily explain why irradiations with heterochromatic light (containing all wavelengths) should have given unpredictable results (Forer, 1966).

**DISCUSSION**

The action spectrum for producing areas of reduced birefringence (ARBs) has peak

![Graph](image)

**Fig. 10.** Curves that show both the stoppage of movement and reduction of spindle fibre birefringence. Percentage of cells with stopped chromosomes (■) or percentage of cells with ARB on the irradiated spindle fibre (□) versus the dose. The EE50 values for movement and ARB production were calculated from these curves as shown. Each graph is for an individual wavelength, as indicated.
sensitivity at 260 nm with a secondary peak at 280 nm (Fig. 8). This is quite different from the action spectrum for blocking chromosome movement that has peak sensitivities at 270 nm and 290 nm (Fig. 11). Thus, at least four different chromophores are involved, two for each effect, and we conclude that movement is sensitive to wavelengths of ultraviolet light different from those that affect birefringence; the two parameters seem to be altered independently. To us, as argued below, the most likely interpretation is that molecular components involved in movement are different from those that give rise to birefringence and that the two different sets of components have different wavelength sensitivities. The alternative interpretation, that there is a single molecular component that has two chromophores involved in birefringence and two different chromophores involved in force production, seems to us to be much less...

Fig. 10C,D. For legend see p. 19.
Fig. 11. Two action spectra on the same scale: the action spectrum for stopping chromosome movement (●) and that for changes in spindle fibre birefringence (○). Note that the two peaks for stopping chromosome movement, at 270 nm and 290 nm, are the troughs for changes in spindle fibre birefringence.

Table 2. Comparison of the EE50 values for stopping chromosome movement with those for changes in spindle fibre birefringence

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>EE50 movement (ergs/μm²)</th>
<th>EE50BR (ergs/μm²)</th>
<th>Ratio of EE50BR to EE50 movement</th>
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<tr>
<td>260</td>
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</table>
likely. In any case, the different chromophores involved in the observed effects have different sensitivities to ultraviolet light (Fig. 11).

The most likely interpretation of our data is that the spindle fibre components responsible for chromosome movement are different from those responsible for birefringence. One reason for this is our expectation that it would take less dose to block force production than to disrupt the structure grossly (i.e. change the birefringence), as in muscle (Stephens, 1965). That one consistently causes gross disruption of the birefringent components — that is, an ARB — without disruption of force (i.e. no stoppage of chromosome movement), simply by shifting to 260 nm wavelength light (Fig. 11), strongly suggests to us that there are two different sets of components involved, the force production components having different u.v. chromophores and being different molecular components from those giving rise to spindle fibre birefringence. Another reason is the expectation that if there were only one component, then in cells displaying stoppage of chromosome movement without a change in birefringence there would be less damage than in those showing stoppage of chromosome movement with a change in birefringence, and that therefore there should be longer stoppage times for chromosomes associated with an ARB than for those with no ARB. This is clearly not the case: when cells are irradiated in anaphase and the irradiation is sufficient to stop chromosome movement we noticed no significant difference between the stoppage time of chromosomes associated with an ARB and those without an ARB. A third reason is that if the ARB represents damage to the force producer then when an ARB is produced and chromosome movement concomitantly stops one would expect movement to resume only after repair of the damage — that is, only after the damaged region, the ARB, reaches the pole. But in all cases when an ARB was produced the movement of the chromosomes resumed before the ARB reached the pole. For all these reasons we feel that the most likely interpretation is that there are at least two sets of components involved, those contributing to force production and those contributing to the birefringence. With knowledge of the action spectra we can reproducibly and consistently produce the desired effect, and this should enable us to test morphologically what has been altered by the irradiation (e.g., using electron microscopy, or reaction with specific antibodies).

It is relevant to point out that since anaphase chromosome movement was unaltered when an ARB was on the spindle fibre, when the anaphase irradiations were of proper wavelength (260 nm) and dose, most of the birefringent material is not necessary for chromosome movement.

We have compared the action spectrum for changes in spindle fibre birefringence with absorption spectra of spindle fibre components; none of these suspected components has both a 260 nm and a 280 nm absorption peak, but several have an absorption peak at 280 nm only, including microtubules (Taniguchi & Kuriyama, 1978), myosin (Caspersson & Thorell, 1942), tubulin (Eipper, 1974) and calmodulin (Kuo & Coffee, 1976; Watterson et al. 1976). Some of our results may be due to effects on one or all of these components, but, as we have argued previously (Sillers & Forer, 1981c), the absorption spectrum averages chromophores over an entire molecule,
Action spectrum for changes in spindle fibre birefringence

whereas there may be some portions of the molecule that are more important or more sensitive than others; absorption by the more sensitive chromophores (i.e. only part of the entire molecule) might cause the effect whilst absorption by other chromophores (i.e. the entire molecule) does not. For example, whereas myosin has a peak absorption at 280 nm (Caspersson & Thorell, 1942), myosin has tryptophan and tyrosine residues near the active site, such that the absorption spectrum for the myosin subfragment S1 has a single peak at 290 nm (Shimizu, Morita & Yagi, 1971); thus 290 nm irradiations would be expected to be very efficient in blocking myosin function. Hence, if we assume that the change in birefringence is due to local depolymerization of microtubules, then the 280 nm peak might be due to absorption by tubulin and the 260 nm peak might be due to absorption by the associated nucleotide, GTP. (While we know of no data on the effects of ultraviolet light on microtubule depolymerization, the most effective u.v. wavelength for blocking microtubule polymerization is 280 nm (Zaremba & Irwin, 1980).) The action spectrum for blocking chromosome movement is parallel to that for blocking myofibril contraction but is quite different from that for blocking ciliary beating (Sillers & Forer, 1981c), and might be due to depolymerization of actin by 270 nm wavelength light and inactivation of myosin by 290 nm wavelength light (see discussion by Sillers & Forer, 1981c).

Metaphase cells responded differently from anaphase cells after u.v. microbeam irradiation: when anaphase started shortly after an irradiation of one or two spindle fibres in metaphase, subsequent poleward movement was blocked for all six half-bivalents, whereas after irradiations during anaphase chromosome movement was blocked only for those chromosomes associated with the irradiated spindle fibre (and their partners). This suggests that the motors of different half-bivalents are linked in some manner in metaphase but that they become independent by anaphase (see also Forer, 1966). The limited data we present on wavelength sensitivity suggest that a chromophore that absorbs light of wavelength 280 nm is responsible for this effect. Regardless of the validity of this conclusion, our data indicate a fundamental difference between metaphase and anaphase cells.

In metaphase cells with an ARB that entered anaphase shortly after the irradiation, anaphase chromosome movement was often normal. On the other hand, Forer (1966) found no such cell (in 15 cases) in which chromosome movement was normal. It seems most likely to us that Forer's (1966) results are due to the use of heterochromatic u.v. light, in that several sensitive chromophores were irradiated at once. By using monochromatic u.v. light we have disentangled the separate effects. We suggest, therefore, that ARB production per se need not alter subsequent anaphase movement, but when a different chromophore is altered (perhaps one that absorbs at 280 nm) then anaphase movement is blocked.

We have quantified the birefringence of individual spindle fibres after irradiations. In irradiations in which we blocked chromosome movement without an apparent change in the birefringence of the spindle fibre we have confirmed that there is indeed no change in the birefringence of either the irradiated spindle fibre or any other spindle fibres in the same cell. Whatever u.v.-sensitive component is responsible for
force production, changes certainly cannot be seen as birefringence changes. In ir-
radiations that produced an ARB, the ARB represented (in four cells) a region in
which 40–70% of the original birefringence was lost, whereas at the same time there
was no change in the birefringence of the rest of that spindle fibre or any other spindle
fibres in that cell. When an ARB is produced, the remaining birefringence might
represent an unaffected portion of the one original birefringent component, or it
might represent a birefringent component that is separate and different from the one
that was destroyed by the u.v. light. It might even represent non-tubulin protofila-
mentous components of microtubules (Linck & Langevin, 1982) that are not affected
by the irradiations.

Leslie & Pickett-Heaps (1981) found lesions in the central spindle of diatoms
caused by ultraviolet microbeam irradiations; there were no microtubules in the lesion
areas. These irradiations, however, were with heterochromatic ultraviolet light, so it
is difficult to ascertain which of the four sensitive chromophores they altered; i.e.,
whether the birefringence components or the movement components, or both, have
been affected by the irradiation. The lesion that they induced in the central spindle
did not move poleward as did those in our experiments, and those of Forer (1965),
on chromosomal spindle fibres; instead the birefringence disappeared polewards from
the poleward edge of the lesion. The difference between their results and ours might
be a species difference, or a difference between continuous fibres and chromosomal
fibres, or might be due to the use of heterochromatic u.v. light that altered several
chromophores at once, and thereby made the physiological outcome different from
our results.

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