PROMETAPHASE FORCES TOWARDS OPPOSITE SPINDLE POLES ARE NOT INDEPENDENT: AN ON / OFF CONTROL SYSTEM IS IDENTIFIED BY ULTRAVIOLET MICROBEAM IRRADIATIONS

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SUMMARY
Individual spindle fibres in prometaphase spermatocytes of the cricket, Neocurtilla hexadactyla, were irradiated with an ultraviolet microbeam. The stretched heteromorphic bivalent (X2Y) contracted to about 75% of its pre-irradiation length after irradiation of either of its two oppositely directed spindle fibres (i.e., the X2 or Y spindle fibre). The X2Y bivalent also contracted after irradiation of the connection between the kinetochores of the univalent Xi chromosome and the Y chromosome but it did not contract after irradiation of autosomal spindle fibres or of the spindle fibre of the Xi univalent sex chromosome. The spindles sometimes shrank after irradiation, but contraction of the X2Y bivalent was independent of spindle shrinkage.

The data strongly suggest that the oppositely directed forces on a bivalent are not independent. One reason is that the X2Y contractions were asymmetrical: during contraction one kinetochore remained fixed in position while the other kinetochore (facing the opposite pole) moved towards the equator. In 75% of the cases the stationary kinetochore was associated with the irradiated spindle fibre. Thus, we suggest that the irradiation of a spindle fibre produces a state analogous to rigor in the irradiated spindle fibre and, because of effects on the control system, produces relaxation of tension in the oppositely directed non-irradiated spindle fibre, so that the kinetochore associated with the non-irradiated spindle fibre moves towards the equator. These experiments have identified a control system that coordinates force production to opposite poles.

INTRODUCTION
The forces for anaphase chromosome movements come from the chromosomal spindle fibres that extend between each chromosome and the spindle pole (see reviews by Nicklas, 1971; Forer, 1969, 1974, 1980; McIntosh, 1979, 1982). These fibres contain microtubules, actin and other components (e.g. see McIntosh, 1979, 1982), but there is no general agreement on which spindle-fibre components produce the force for chromosome movement (e.g. see Inoué, 1981; Dietz, 1972; Gruzdev, 1972; McIntosh, Hepler & Van Wie, 1969; Nicklas, 1975; Margolis & Wilson, 1981; Forer, 1974, 1981; Forer, Jackson & Engberg, 1979). The forces for prometaphase chromosome movements are thought to come from the chromosomal spindle fibres and to be produced by mechanisms similar to those that produce the force for anaphase chromosome movements, but there is no direct proof of this. We set out to test whether prometaphase force production is similar to anaphase force production:
we irradiated individual chromosomal spindle fibres to see if the wavelength sensitivities for blocking force production in prometaphase and in anaphase are the same.

In anaphase, when individual chromosomal spindle fibres are irradiated with an ultraviolet microbeam both the associated half-bivalent and the partner half-bivalent (going to the opposite pole) stop moving, temporarily, for 3–10 min (Sillers & Forer, 1981c; Forer, 1966). The most effective wavelengths for blocking anaphase force production are 270 nm and 290 nm (Sillers & Forer, 1981c). To see if prometaphase force production is similar to anaphase force production, one can irradiate chromosomal spindle fibres in prometaphase, obtain an effect, and see if the wavelength sensitivity for that effect is the same as that for blocking anaphase chromosome movement (namely, peaks at wavelengths 270 and 290 nm). How does one determine if there is an effect of the ultraviolet light on the prometaphase force producer?

During the course of other experiments with mole cricket spermatocytes (Wise, Sillers & Forer, unpublished), we found that ultraviolet microbeam irradiations blocked prometaphase force production: the stretched heteromorphic X2Y bivalent (Payne, 1912, 1916; White, 1951; Camenzind & Nicklas, 1965; Kubai & Wise, 1981) contracted when either of its chromosomal spindle fibres was irradiated. The change from 'stretched' to 'non-stretched' must be due to a reduction in the force applied to the prometaphase chromosome, and therefore this 'contraction' can be used to assay the effects of the ultraviolet microbeam irradiation on prometaphase force production. We report here the results of ultraviolet microbeam experiments in which we investigated the wavelength dependence for causing contraction of the X2Y bivalent.

MATERIALS AND METHODS

Animals and cells

*Neocurtilla hexadactyla* (formerly called *Gryllotalpa hexadactyla*) individuals were obtained as described by Wise *et al.* (unpublished), and living spermatocyte preparations were made as described therein. Briefly, follicles were pierced under halocarbon oil (Forer, 1982) and the cells were spread, under the oil, onto a quartz coverslip that was taped over a hole in a metal slide (described by Sillers & Forer, 1981a). The ultraviolet microbeam microscope is a modified Nikon model M inverted microscope, and the optical layout and the procedures for dosimetry, for recycling quartz coverslips, for irradiation using phase-contrast microscopy lenses, and for cinematography and videotape recording of data have been described elsewhere (Sillers & Forer, 1981a,b, 1983). For all the experiments reported in this paper the ultraviolet microbeam was focussed to a circle 4·2 μm in diameter, and irradiation times were between 5 s and 40 s, giving total doses of 0·01–0·14 ergs/μm². Data used in this paper were recorded on both videotape and 16 mm film, but analysis was primarily from videotape images. *Neocurtilla* spermatocytes have been described elsewhere (Payne, 1912, 1916; White, 1951; Camenzind & Nicklas, 1965; Kubai & Wise, 1981; Wise *et al.* unpublished): the large partner of the X2Y (heteromorphic) bivalent always moves to the same spindle pole as does the univalent X1 chromosome. The chromosomal spindle fibres are of the order of 1 μm in diameter (Kubai & Wise, 1981).

Analysis of videotape data

'Contraction' of the X2Y bivalent is defined operationally as a change in length of the bivalent after the irradiation. We are able to detect length changes of 10% or more, and contraction was analysed in several ways from videotape images that were viewed on a TV monitor. First, all three of us
u.v. microbeam irradiations in prometaphase

observed each cell before and after an irradiation, using normal speed play back; the evaluations of contraction were made 'blind', without any of us looking at the protocols to see which particular spindle fibre was irradiated and each of us scored independently whether the X2Y bivalent did or did not contract. There were very few disagreements: in most cases it was very obvious whether or not the X2Y bivalent contracted. We reanalysed those on which we disagreed (using tracings on acetate sheets and frame-by-frame analysis) and reached agreement. The analysis by eye was repeated (after a long time interval) by two of us (P.J.S. and D.W.); there were no discrepancies. These two analyses gave information on which irradiations caused contractions. For detailed information on the contractions themselves, single frames were viewed on the TV monitor and we traced on acetate sheets outlines of each cell, the spindle, the autosomes, the X1 sex-chromosomal univalent, and the X2Y bivalent. For all of the cells we made at least three tracings from each cell: one was just prior to the irradiation, one was immediately after the irradiation, and one was 10 min after the irradiation. From the tracings we deduced which side of the X2Y bivalent actually moved during contraction, and how much the bivalent contracted (i.e., the initial length of the stretched bivalent versus the length of the contracted bivalent). For selected cells we made measurements at 1-min intervals to determine rates of contraction and elongation. The measurements were made directly from the video images, either from tracings or from measurements taken directly from the video screen.

Data presented in Results are given as average values±standard deviations.

RESULTS

We irradiated different chromosomal spindle fibres in different cells, but only one spindle fibre per cell. If one considers the univalent chromosome to be the X1 (sex) chromosome, the large partner of the X2Y bivalent to be the X2 chromosome, the small partner of the X2Y bivalent to be the Y chromosome (White, 1973; Kubai & Wise, 1981) and all other bivalents to be autosomes, in any given cell we irradiated either an autosomal spindle fibre, the X1 spindle fibre, the X2 spindle fibre, or the Y spindle fibre, as illustrated in Fig. 1. In six cells we irradiated in a direct line between the kinetochores of the X1 chromosome and the Y chromosome, to aim for the connection that extends between the two kinetochores. (These connections, of much smaller diameter than a chromosomal spindle fibre, are composed of only a few microtubules; Kubai & Wise, 1981.) We irradiated the chromosomal spindle fibres with the edge of the irradiating spot about 2 μm away from the kinetochore. In eight cells we irradiated at one pole; the results of these irradiations were no different from irradiations of X2 or Y spindle fibres, so we pooled the data from the 'pole' irradiations together with those of the X2 or Y spindle fibres, respectively.

It is important to recognize that ultraviolet light can kill cells, and that some of the effects observed in any given ultraviolet microbeam experiment might be 'non-physiological', lethal effects of the irradiation. In the experiments reported here (and by Wise et al. unpublished) none of the effects of the irradiations are degenerative changes. We say this because the morphology of cells and chromosomes did not change after irradiations and because the effects of the irradiations were reversible, as described below. Furthermore, cells entered and completed normal anaphase after irradiations with the highest doses that we used. We did not make a point of filming each cell that we irradiated from the time of the irradiation until anaphase started, but anaphase sometimes started shortly after the irradiation. This happened after four irradiations, and in each such cell anaphase appeared to be normal (e.g. see Fig. 2);
Fig. 1. This schematic drawing (which is traced from an actual cell) shows a typical mole cricket spermatocyte. The $X_1$ univalent, the $X_2Y$ bivalent and the autosomes are all indicated. The different sites of irradiation are marked by circles and numbers. Although the cell is drawn to scale the sites of irradiation are half their actual size (i.e. the irradiation sites are $2\mu m$ on this drawing whereas they are actually $4\mu m$). The irradiations were classified as follows: 1, $X_1$ fibre irradiation; 2, $X_2$ fibre irradiation; 3, $Y$ fibre irradiation; 4, autosomal fibre irradiation (either on the $Y$ side or the $X_2$ side); 5, $X_1$–$Y$ connection irradiation. Irradiations at either the $X_2$ pole or the $Y$ pole were pooled together with either $X_2$ fibre irradiations or $Y$ fibre irradiations, respectively, as described in the text.

Fig. 2. A series of pictures showing a cell that was irradiated shortly before anaphase. In this cell the $X_2$ spindle fibre was irradiated with a dose of 0.04 ergs/μm² and 290 nm wavelength light. Just after the irradiation (a) the $X_2Y$ bivalent contracted a slight amount but then the cell immediately went into anaphase. The times of the photos are: a, 0 min (just prior to the irradiation); b, 2 min; c, 4 min; d, 11 min. ×1300.

the chromosomes moved at about the same velocity as did chromosomes in non-irradiated cells in the same preparations. Thus the effects of the irradiations that we describe below are physiological responses to the ultraviolet light and are not just degenerative changes due to onset of cell death.

The $X_2Y$ bivalent shortened in length (contracted) after irradiation of either the
u.v. microbeam irradiations in prometaphase

Table 1. Overall effects of the irradiations on contraction of the X2Y bivalent

<table>
<thead>
<tr>
<th>Site of irradiation</th>
<th>No. of irradiations</th>
<th>No. of times X2Y contracted</th>
<th>% of irradiations in which X2Y contracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>X2 spindle fibre</td>
<td>36</td>
<td>16</td>
<td>44</td>
</tr>
<tr>
<td>Y spindle fibre</td>
<td>39</td>
<td>21</td>
<td>54</td>
</tr>
<tr>
<td>X1-Y connection</td>
<td>6</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>Overall</td>
<td>81</td>
<td>41</td>
<td>51</td>
</tr>
<tr>
<td>X1 spindle fibre</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Autosomal spindle fibre</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

X2 or Y spindle fibres and after irradiation between X1 and Y. The X2Y bivalent did not contract after any of seven irradiations of autosomal spindle fibres, or after any of six irradiations of X1 spindle fibres. No other chromosomes contracted after irradiation of any spindle fibres or the poles and thus the contraction was specific to the X2Y bivalent and further specific to irradiation of either an X2 or Y spindle fibre or the X1-Y connection. The X2Y bivalent contracted about half the time (41 out of 81 irradiations) when irradiated in either the X2 or Y spindle fibre or in the X1-Y connection (Table 1).

In considering these results one would like to know how well the ultraviolet microbeam was aimed. In earlier experiments (Sillers & Forer, 1981a, b) we deduced that in our system the visible image of the irradiating spot is exactly coincident with that of the ultraviolet light, so there is little if any error in this regard. However, when we focus the 4 μm diameter spot to one site in a cell the spot might also irradiate other sites in that cell, depending on the relative positions of the spindle fibres in question. For example, ultraviolet light focussed to a 4μm diameter circle and centred on the X2 spindle fibre of Fig. 1 (position 2 in Fig. 1) would also irradiate the X1-Y connection. In order to see if this possible error might alter our interpretations we carefully analysed the recorded images of each cell to see which irradiations might have resulted in irradiations of two fibres instead of only the one we were aiming at; the results of this analysis are as follows. Out of all of the irradiations of X2 spindle fibres (see Table 1), we might have irradiated the X1-Y connection in five cells and we might have irradiated the X1 spindle fibre in 12 other cells; the X2Y bivalent contracted in two cases out of the five (in which there possibly was co-irradiation of the X1-Y connection) and the X2Y bivalent contracted in five cases out of the 12 (in which there possibly was co-irradiation of the X1 spindle fibre). (The latter case, co-irradiation of the X1 fibre, does not introduce any ambiguities into the interpretation of the results since, as already mentioned, direct irradiations of X1 spindle fibres do not cause the X2Y bivalents to contract (Table 1).) Out of all of the irradiations of X1-Y connections (see Table 1), we might have irradiated the X2 spindle fibre in one cell; the X2Y bivalent contracted in this cell. In all other cells the positions of the chromosomes and spindle fibres were such that we are certain that we irradiated only the fibre that we aimed at, and not others. In conclusion, some of the irradiations were indeed of
two sites at once, which results in some ambiguity (in knowing, e.g., whether contraction of the X₂Y bivalent is due to irradiation of the X₂ spindle fibre or to irradiation of the co-irradiated X₁-Y connection). It is clear from the numbers summarized above, however, that only a small fraction of cases (summarized in Table 1) have this ambiguity, and that these small numbers of possibly ambiguous cases do not alter the overall interpretations.

The contraction of the X₂Y bivalent was primarily in the pole-to-pole direction regardless of which spindle fibre (X₂ or Y) was irradiated or whether the X₁-Y connection was irradiated: in only three out of the 41 contractions did the X₂Y bivalent appear to move laterally, in which cases both kinetochores moved laterally. (These exceptional cases were after irradiation of X₂ or Y spindle fibres.) The X₂Y bivalents that contracted did so to about 75% of their initial length (Fig. 3): the average was 72.6 ± 7.7% (n = 37). The contraction was to the same extent regardless of whether the X₂ fibre (n = 16), the Y fibre (n = 21), or the X₁-Y connection (n = 4) was irradiated (Fig. 4).

We followed the timing of the contraction. Either the X₂Y bivalent contracted during the irradiation and was completely contracted before we could look at it (n = 20), which was within 5 s after completion of the irradiation, or the bivalent did not begin to contract until 1–2 min after the irradiation (n = 21). In two cases only, the bivalent began to contract during the irradiation and continued to contract afterwards. The average rate of contraction for all bivalents (including the two that started to contract during the irradiation) was 0.45 ± 0.13 μm/min (n = 23). Whether the contraction was 'immediate' or 'delayed' seemed to occur at random, and, as deduced from curves and tables, did not seem to be a function of wavelength, dose, the region that was irradiated, or the asymmetry of the contraction that is described below.

Fig. 3. A histogram showing the number of X₂Y bivalents that contracted versus the amount of contraction, expressed as a percentage of the pre-irradiated length of the X₂Y bivalent. Only the X₂ and Y fibre irradiations are included in this graph.
The contraction was not permanent: the X2Y bivalent eventually re-extended. The length of time that the bivalent remained contracted before re-extension seemed to be greater for those bivalents that contracted immediately than for those with delayed contraction; we generally stopped recording the cells 10 min after the irradiation, so the distribution of times that the bivalents remained contracted ranges from 2 min to ‘more than 10 min’ (Fig. 5). If we assume that each bivalent that remained contracted for more than 10 min in fact began to re-extend at 12 min, the overall average time before re-extension (Fig. 5) was 8.1 ± 3.4 min; for immediate contractions (Fig. 6A) the corresponding average was 9.3 ± 3.2 min (n = 20), whereas for delayed contractions (Fig. 6B) the average was 6.9 ± 3.5 min (n = 21), a difference that is statistically significant (using Student’s t-test) at about the 0.03 level. We measured the speed of
Fig. 5. A histogram showing the number of X2Y bivalents that contracted versus the length of time (in min) that the X2Y bivalent remained contracted. This graph includes irradiations of X2 spindle fibres, Y spindle fibres and X1-Y connections. If one considers that >10 min is equal to 12 min, then the average time (±s.d.) that an X2Y bivalent remained contracted was 8.1 ± 3.4 min (n = 41).

re-extension of four contracted bivalents (e.g. see Fig. 7): these averaged 0.28 ± 0.5 μm/min (n = 4), about half the speed at which the bivalents contracted.

There was an asymmetry to the contraction of the X2Y bivalent: in most cases the kinetochore on the side opposite to the irradiation moved towards the equator (contracted) while the kinetochore on the irradiated side remained fixed in position (Fig. 8). Of the 41 cells in which the X2Y bivalent contracted: in one cell the bivalent contracted from both sides, in nine cells the bivalent contracted from the same side as the irradiation, and in 31 cells the bivalent contracted from the side opposite that of the irradiation. (These data include 'opposite side' contraction when the X1–Y connection was irradiated: in all four cells in which the X2Y bivalent contracted after irradiation of the X1–Y connection the X2 kinetochore moved towards the stationary Y kinetochore.) That is to say, 70% of the time the contraction was from the side opposite to the side that was irradiated. The amount of contraction was the same regardless of whether contraction was from the side irradiated or from the opposite side (Fig. 9). Also, as deduced from tables and graphs, there was no correlation between the sidedness of the contraction of the X2Y bivalent and whether, as described above, the contraction was immediate or was delayed.

The irradiations sometimes caused the spindles to shrink, i.e., caused the spindle pole-to-pole distance to decrease. (The spindles shrunk either symmetrically, both poles moving equal distances towards the equator, or asymmetrically, with one pole
Fig. 6. Two histograms showing the number of X₂Y bivalents that contracted versus the length of time (min) that the X₂Y bivalents remained contracted. The histograms have been separated into those X₂Y bivalents that contracted immediately (A), and those X₂Y bivalents with delayed contraction (B), as described in the text. If one considers that >10 min is equal to 12 min then the average time (±S.D.) that an immediate X₂Y bivalent remains contracted is 9.3 ± 3.2 min (n = 20), whereas the average time that a delayed X₂Y bivalent remains contracted is 6.9 ± 3.5 min (n = 21).

remaining a fixed distance from the equator while the other pole moved towards the equator.) The following analysis shows that spindle shrinkage was not causally related to contraction of the X₂Y bivalent.

There was spindle shrinkage about 50% of the time after irradiation of autosomal spindle fibres, X₁ spindle fibres, X₂ spindle fibres, Y spindle fibres or the connection between X₁ and Y chromosomes. The X₂Y bivalent contracted in 41 cells after an irradiation; we have data on spindle shrinkage in 35. Of these, there was spindle shrinkage in 20 and there was no change in spindle length in the other 15 (Table 2).
Fig. 7. This graph shows the length of an X2Y bivalent before and after an irradiation versus time. The black bar represents the time when the irradiation occurred. This particular cell was irradiated with 290 nm wavelength light at a dose of 0.1 ergs/μm². The X2Y bivalent contracted at a rate of 0.65 μm/min, remained contracted for 2.5 min, and then re-extended at a rate of 0.25 μm/min.

Fig. 8. This series of pictures shows a stretched X2Y bivalent (arrow), before the irradiation, that contracted after the irradiation. In this cell the X2 spindle fibre was irradiated using a dose of 0.04 ergs/μm² and 290 nm wavelength light. Note that the side opposite to the irradiation (the Y side) was the side of the chromosome that moved. In this particular cell the spindle did not shrink and the X2Y bivalent began to re-extend. The times are: A, 0 min (just prior to the irradiation); b, 3 min; c, 4 min; d, 12 min. ×1000.

The X2Y bivalent did not contract in 45 cells after an irradiation; we have data on spindle shrinkage in 39, and of these there was spindle shrinkage in 15 and no change in spindle length in the other 24 (Table 2 and Fig. 10). Thus spindle shrinkage occurred 57% of the time when the X2Y bivalent contracted (n = 35) and 38% of the time when the X2Y bivalent did not contract (n = 39). Furthermore, the sidedness of shrinkage of the spindle is not related to the sidedness of contraction of the X2Y bivalent; there are 10 cells for which we have data in which there is both asymmetric spindle shrinkage and contraction of the X2Y bivalent, and in four of these the spindle
shrinkage was on the side opposite to that of the X2Y contraction. Finally, when the spindle shrank at the same time that the X2Y bivalent contracted, the bivalent re-extended to its original length, whereas in the same cells the pole-to-pole distance of the spindle did not re-extend after shrinkage (Fig. 11) and remained at the same short length \((n = 2)\). Thus, for all these reasons we conclude that contraction of the X2Y bivalent is not due to shrinkage of the spindle: the two are independent events, as shown by every measure that we have used.

Contraction of the X2Y bivalent was caused equally well by irradiation of the X1–Y connection or the X2 spindle fibre or the Y spindle fibre, as seen in Table 1.
Fig. 10. This series of pictures shows a cell in which the X₂Y bivalent did not contract but the spindle shrank. The X₂Y bivalent is indicated by an arrow. This bivalent was irradiated on the X₂ spindle fibre side with 290 nm wavelength light at a dose of 0·05 ergs/μm². The times are: a, 0 min; b, 2 min; c, 5 min. ×1200.

Fig. 11. This series of pictures shows an X₂Y bivalent stretched (arrow) before the irradiation of the X₂ spindle fibre and contracted after the irradiation; the X₂Y bivalent contracted from the Y side (the opposite side) to 75 % of its original length. (The irradiation was with 290 nm wavelength light at a dose of 0·01 ergs/μm².) The X₂Y bivalent re-extended and after 12 min had reached its original length; the spindle did not elongate after the irradiation, thus confirming that the two events, X₂Y bivalent contraction and spindle shrinkage, are independent. The times are: a, 0 min (just prior to irradiation); b, 3 min; c, 5 min; d, 7 min; e, 13 min. ×1300.

Table 3. Wavelength effects on the sidedness of the X₂Y contractions

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>X₂ Same</th>
<th>X₂ Opposite</th>
<th>Y Same</th>
<th>Y Opposite</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>280</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>270</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>260</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>4</strong></td>
<td><strong>12</strong></td>
<td><strong>5</strong></td>
<td><strong>16</strong></td>
</tr>
</tbody>
</table>

We studied the effectiveness of different wavelengths in causing the X₂Y bivalent to contract. There was no correlation between increased dose and increased effect at any wavelength. Thus, it was impossible to determine an action spectrum for causing contraction of the X₂Y bivalent. The data showing the effects of wavelength and dose on contraction of the bivalent are given in Fig. 12; there was no difference between
Table 4. Wavelength effects on the sidedness of the X2Y bivalent contraction

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Same side contracts</th>
<th>Opposite side contracts</th>
<th>Both sides contract</th>
</tr>
</thead>
<tbody>
<tr>
<td>270, 290</td>
<td>8</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>260, 280</td>
<td>1</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>9</strong></td>
<td><strong>28</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>

Fig. 12. At each wavelength (260, 270, 280, 290 nm) we have plotted % X2Y bivalents that contracted versus the dose, for irradiations of X2 and Y spindle fibres only. (The six irradiations of the X1–Y connection were all of 0-1 ergs/μm², and resulted in four contractions of the X2Y bivalent.) In these curves we have pooled the data from different doses: 0-01–0-06 ergs/μm² data were pooled and plotted as 0-04 ergs/μm²; 0-08–0-12 ergs/μm² data were pooled and plotted as 0-1 ergs/μm², and 0-13–0-15 ergs/μm² data were pooled and plotted as 0-14 ergs/μm²; n is the number of irradiations. It is clear that increased dose does not have an increased effect on X2Y bivalent contraction; because there is no exponential increase of effect with dose we are unable to obtain an action spectrum.

irradiating X2 spindle fibres and irradiating Y spindle fibres, so in this analysis these data were pooled.

The asymmetry (the sidedness) of the contractions of the X2Y bivalents did depend on wavelength, however, as shown in Tables 3 and 4, in which we have pooled results from irradiations of X2 or Y spindle fibres from all doses: the contractions occurred only on the side opposite to the irradiation in 14 out of 15 cases with 260 and 280 nm wavelength irradiations, but in 14 out of 23 cases with 270 and 290 nm wavelength...
irradiations. That is, using ultraviolet light of wavelengths 260 and 280 nm, almost no contractions of the bivalent take place on the same side as the irradiations (only about 7 %), but using ultraviolet light of wavelengths 270 and 290 nm large numbers of contractions are on the same side as the irradiation (36 %, or 39 % if one includes the one contraction that was from both sides).

**DISCUSSION**

The stretched X2Y bivalent in *Neocurtilla* spermatocytes contracted after ultraviolet microbeam irradiation. The contraction was induced by irradiation of either of the two chromosomal spindle fibres associated with the bivalent, or by irradiation of the connection between the Y kinetochore and X1 kinetochore (Kubai & Wise, 1981). The effect is specific for those sites of irradiation: there were no contractions when chromosomal spindle fibres of either the autosomes or the X1 chromosomes were irradiated. The contraction is not due to a generalized effect on the spindle because contraction of the X2Y bivalent is independent of spindle shrinkage: the bivalent contracted even when the spindle did not shrink, and the spindle shrank even when the bivalent did not contract. Even when spindle shrinkage occurred at the same time that the bivalent contracted the bivalent re-extended without re-extension of the spindle. Thus the contraction of the X2Y bivalent is an effect specific to ultraviolet microbeam irradiation of the associated spindle fibres (i.e., of the X2 or Y chromosomal spindle fibres or the X1–Y connection) and is not due to general effects on other spindle components.

The ultraviolet microbeam irradiations that cause the X2Y bivalent to contract most likely alter a control system, or a 'switch', rather than directly altering the force production system itself. Consider a case in which either the X2 or Y chromosomal spindle fibre was irradiated. If the ultraviolet light were to alter only the force-producing system, then the force producer on the irradiated side would be turned off while the force producer on the non-irradiated side would be unaffected. In that case one would expect that the half-bivalent on the irradiated side would contract (i.e., that the kinetochore would move toward the equator) while the entire bivalent moved towards the pole on the non-irradiated side because of continued force production by the fibre on the non-irradiated side (Hays, Wise & Salmon, 1982). This never occurred and most of the time the 'wrong' kinetochore contracted: in 75 % of the cells in which the X2Y bivalent contracted the kinetochore associated with the irradiated fibre stayed in place, without moving at all, while the kinetochore oriented to the opposite pole contracted (moved toward the equator). That is, the contraction occurred as if the kinetochore associated with the irradiated spindle fibre were held in place by a spindle fibre that allowed no movement, either toward the pole or away from the pole, as in 'rigor', while at the same time the force on the other, oppositely directed kinetochore was turned off in a 'relaxed' state with a spindle fibre capable of elongating; when both forces were turned off the tension in the stretched chromosome caused the contraction. The only explanation we can think of for this result is that the microbeam irradiation of a chromosomal spindle fibre altered a control system, such
as a switch in an on/off signalling device, such that a signal is sent to the oppositely
directed chromosomal spindle fibre to cause the force to be turned off; when the force
is turned off in this way it is turned off in a relaxed state, unlike the irradiated fibre
that is turned off in rigor. That is to say, the two oppositely directed prometaphase
force production systems are not independent, and irradiations of a control system
have effects on both spindle fibres. In those cases in which the contraction was of the
kinetochore on the same side as the irradiation, i.e. 25% of the cells with an effect,
the kinetochore on the opposite side did not move poleward at all. Thus, according
to our interpretation, these irradiations must have stopped force production on the
irradiated side in the relaxed state while the force producers on the other, opposite,
non-irradiated side must have been altered by a switch and turned off in rigor;
otherwise the bivalent would have moved towards the pole on the non-irradiated side.
In those irradiations of the X1–Y connections that caused contraction of the X2Y
bivalent, the X2 kinetochore was the one that moved. According to our interpretation,
then, this is due to the irradiation causing force production from the Y spindle fibre
to be turned off in rigor while the force producers in the X2 spindle fibre were turned
off in a relaxed state, perhaps because the microtubules (and associated components)
in the X1–Y connection continue directly into the Y spindle fibre (Kubai & Wise,
1981). We conclude, then, that the ultraviolet-microbeam-induced contraction of the
X2Y bivalent in mole cricket spermatocytes is due to effects on both the force produc-
tion systems and control systems that regulate forces to both poles.

Several questions are raised by these conclusions. One concerns the generality of
the results. Neocurtilla spermatocytes clearly have unique control systems that
generate non-random segregation of chromosomes. Is our conclusion that the irradia-
tions alter a control system restricted to this specialized, unique cell type? This
conclusion is not unique, and similar effects can be seen in several other cases, as
follows. When an individual chromosomal spindle fibre in crane-fly spermatocytes is
irradiated in anaphase the poleward movement of the associated chromosome is
stopped, with no effect on other chromosomes moving to the same pole. However, the
partner chromosome (with which the stopped chromosome was previously joined)
also stops moving to the opposite pole (Forer, 1966; Sillers & Forer, 1981c, 1983). The
poleward movements of both partners resume at the same time. In this case too, then,
the force production systems that pull towards opposite poles are not independent.
(As an extension of the interpretation of the results in this paper, we predict that after
ultraviolet microbeam irradiation of anaphase crane-fly spermatocytes one of the
stopped half-bivalents would be in rigor, and not movable away from the pole,
whereas the other half-bivalent would be relaxed, and movable (by micromanipula-
tion) away from the pole.)

Another example of coordinated movements also is found in crane-fly spermatocytes:
irradiation of individual autosomal spindle fibres just prior to anaphase blocks
the poleward movement of all six half-bivalents, even though only one chromosomal
spindle fibre was irradiated (Forer, 1966; Sillers & Forer, 1983); this result is quite
different from irradiations in anaphase, in which the chromosome pair associated with
the irradiated spindle fibre is the only one that stops moving, and indicates that prior
to anaphase the force producers on all half-bivalent kinetochores are interdependent (see Discussion of Sillers & Forer, 1983). In yet another example in crane-fly spermatocytes, ultraviolet microbeam irradiation of an autosomal spindle fibre alters a control system that regulates the subsequent movements of the two sex chromosomes: the irradiation either blocks movement of one sex chromosome (an 'on/off switch?') or causes the direction of motion to be altered (a directionality switch?), depending on the geometry of the irradiated autosomal spindle fibre relative to the sex-chromosomal spindle fibres (Sillers & Forer, 1981a). Thus in crane-fly spermatocytes there are several examples of control systems for interdependent force production.

Another example is seen in grasshopper spermatocytes: after ultraviolet microbeam irradiation of chromosomal spindle fibres in prometaphase/metaphase the associated bivalent contracted to about 75% of the pre-irradiation length (Izutsu, 1959, 1961; Takeda & Izutsu, 1960). In this case, as in Neocurtilla spermatocytes, the contraction was from one side only: the kinetochore on the non-irradiated side 'contracted' (moved towards the equator) while the kinetochore on the irradiated side did not move either towards the pole or towards the equator. (These conclusions were not mentioned by the authors but were deduced from the illustrations of two relevant cells: we traced the illustrations onto transparent paper and our conclusions are based on comparisons of the pre-irradiation and post-irradiation illustrations. Specifically, we studied fig. 4 of Izutsu, 1959 (that is identical to fig. 15 of Izutsu, 1961) and fig. 15 of Takeda & Izutsu, 1960 (that is identical to fig. 14 of Izutsu, 1961). It is relevant to note that other deductions from these two cells confirm exactly our conclusions from experiments on mole cricket spermatocytes: contraction of the bivalent is independent of shrinkage of the spindle (because in one of the two cells the spindle did not shrink); bivalents stretch out to their original length after contraction; the return to pre-irradiation length (in the cell illustrated in fig. 4 of Izutsu, 1959) occurred within the same time period as in Neocurtilla spermatocytes, namely within 20 min (there are no illustrations between 3 min and 40 min in the other cell, so one cannot tell when the original length was achieved); and when the spindle has shrunk due to the irradiation the re-extension of the bivalent occurs without concomitant increase in the spindle pole-to-pole distance.)

We conclude, therefore, that the interpretations and results from the experiments reported herein are general ones, and not restricted to Neocurtilla spermatocytes.

A second question concerns the wavelength requirements for causing contraction: there is no clear action spectrum for this effect. For irradiations in crane-fly spermatocytes that block anaphase chromosome movements, at any one wavelength there is a clear increased effectiveness with increased dose: the dose versus effect curve is exponential (Sillers & Forer, 1981c). Similarly, clear exponential curves are obtained for blocking myofibril contraction and ciliary beating (Sillers & Forer, 1981c), for destroying the entire spindle (Brown & Zirkle, 1967) and for locally reducing spindle fibre birefringence (Sillers & Forer, 1983). But in inducing contraction of the X;Y bivalent in Neocurtilla spermatocytes there is no clear increase in the incidence of contraction with increased dose (Fig. 12); consequently there is no clear action spectrum. We think that this is because several processes are involved in the effect.
It is possible, for example, that contraction could be caused by blocking force production associated with the irradiated spindle fibre, as well as by altering (let us say) two different control systems, one that controls force production of the irradiated spindle fibre (an on/off switch) and another that sends signals to the non-irradiated spindle fibre. Whereas each alteration in itself might cause contraction, with an exponential curve of effect versus dose for each individual component, our experiments cannot distinguish between the different components: we just measure the final result (contraction). Thus if the three different components have different wavelength sensitivities one might not get clear-cut results in dose versus effect curves over the limited dose range we have used. (One should be able to test this interpretation by measuring contraction in a large number of cells, over a large range of doses; it is relevant to note, however, that the animals and cells are very scarce, and we could not attempt as extensive a series of experiments on this cell system as would be needed to test this interpretation.) Thus we think that there is no clear-cut action spectrum or dose dependence because there are several possible systems that can be altered, all of which would give the same final result, contraction of the X2Y bivalent.

Despite the absence of a bona fide action spectrum there may be one hint that the force production system in prometaphase is indeed the same as in anaphase. That hint is that irradiations with different wavelengths had different effects on the sidedness of the contractions: after irradiations with ultraviolet light of wavelengths 260 and 280 nm essentially all contractions were from the side opposite to the irradiation (14 out of 15 cells), whereas after irradiations with ultraviolet light of wavelengths 270 and 290 nm 36% of the contractions (8 out of 22 cells) were from the irradiated side (Tables 1, 2). Since the most effective wavelengths for blocking force production in anaphase (i.e., for stopping anaphase chromosome movement) are 270 and 290 nm (Sillers & Forer, 1981c), these results suggest that irradiations with 270 and 290 nm wavelength light block the prometaphase force producer and the kinetochore on the irradiated side moves towards the equator (contracts), whereas 260 and 280 nm wavelength light does not block the force producer, but rather primarily alters a control system, or control systems, after which the opposite kinetochore contracts. (In all cases a signal of some kind would cause the force producer to hold the non-contracting kinetochore in place, in rigor.)

A final question concerns the identity of the postulated control systems. As discussed above, it seems likely to us that there is a control system for each chromosomal spindle fibre as well as a system that sends signal(s) to oppositely directed fibres (and/or control systems). We do not know what these control systems are, but it is tempting to consider that membrane systems and calcium pumps are somehow involved (e.g. see Hepler, 1977, 1980; Wolniak, Hepler & Jackson, 1980; Jackson & Doyle, 1982). Our data are relevant to theories of force production during prometaphase, most of which assume that the force on each kinetochore is independent of forces on other kinetochores. For example, it has been argued that the final metaphase position in the spindle is a balance of forces to opposite poles and, therefore, assuming that the force exerted by a spindle fibre is proportional to the length of that fibre, one predicts that metaphase chromosomes will be off the equator.
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if there are different numbers of chromosomal spindle fibres directed to opposite spindle poles (e.g. see discussion of Hays et al. 1982). The data presented herein suggest that the forces on a bivalent that are directed to opposite poles are not independent, and that this interdependence of forces may be a complicating factor in interpreting experiments on 'balance of forces' (also see Nicklas, Kubai & Hays, 1982). At least, theories of prometaphase chromosome movements and spindle organization should account for this interdependence.

Ultraviolet-light-induced alterations to control systems were not permanent: these were repaired (as evidenced by re-extension of the bivalents), usually within 10 min (Fig. 5). Similarly, bivalents in grasshopper spermatocytes returned to their original length within 20 min (Izutsu, 1959, 1961), at least in the one cell for which sufficient data were published to allow us to decide on timing. Yet again, chromosomes that stopped moving in anaphase (due to ultraviolet microbeam irradiation of spindle fibres) started moving again within 4–10 min (when irradiated with monochromatic light; Sillers & Forer, 1981b,c) or 10–30 min (when irradiated with heterochromatic light; Forer, 1966). All these examples illustrate the dynamic nature, the 'lability/repairability' of the force production and control systems. Some ultraviolet-light-induced alterations in the spindle control systems do not seem to be repaired, though (Sillers & Forer, 1981a).

The X₂Y bivalent contracted slowly: the rate of contraction was about 0.45 μm/min. If the force was just turned off, immediately, we would have expected the chromosome to contract at a much faster rate than this. For example, Nicklas & Staehly (1967) used a glass micromanipulator needle to stretch bivalents in grasshopper spermatocytes, and when the needle was removed the bivalents contracted to their original length in less than 4 s. The contraction of the X₂Y bivalent in Neocurtilla was at least two orders of magnitude slower than that observed by Nicklas & Staehly (1967). We do not know why the contractions we observed were so slow.

After ultraviolet microbeam irradiation of a chromosomal spindle fibre the X₂Y bivalent in mole cricket spermatocytes contracted to about 75 % of its original length (Figs 3, 4). In three grasshopper spermatocytes irradiated at spindle fibres or poles (Izutsu, 1959, fig. 4; Izutsu, 1961, figs 3, 14; Takeda & Izutsu, 1960, figs 3, 15) the bivalents contracted to about 85 % of their initial length. Similarly, chromosomes that were removed from grasshopper spermatocytes spindles by micromanipulation contracted to about 85 % of their initial length (Nicklas & Koch, 1969, fig. 3) or to about 75 % of their initial length (Nicklas, 1967, figs 1, 5). We conclude, therefore, that prometaphase/metaphase chromosomes are under sufficient tension to stretch the chromosomes by about 20–33 % of the unstretched length, depending on the elastic coefficient of the chromosome. Given that one knew how much force it took to stretch a chromosome, one could then directly deduce how much force was produced by the prometaphase/metaphase chromosomal spindle fibre.

Finally, we were not successful in testing whether force production in prometaphase has the same radiation sensitivity as force production in anaphase. One could directly test whether this is so by irradiating prometaphase spindle fibres to block prometaphase chromosome movements, as a direct comparison to the experiments in
which the irradiations blocked anaphase chromosome movements (Sillers & Forer, 1981b,c).

This work was supported by grants from the W. Garfield Weston Foundation (Toronto), the Natural Sciences and Engineering Research Council of Canada, and the NIH (grant no. GM 28660 to D.W.).

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(Received 17 March 1983–Accepted 10 May 1983)