Effects of Ultraviolet Microbeam Irradiations on Mitosis Studied in *Tradescantia* Cells *in vivo*

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Introduction

In experimental cytology, a technique to attack chemically or physically a desirable part of a mitotic element has long been sought. Recently, however, through the device of microbeam irradiation, this intention has become possible to irradiate a particular area of mitotic figures of less than few microns in diameter (Uretz, Bloom and Zirkle 1954, Bloom, Zirkle and Uretz 1955, Gaulden and Perry 1958, Izutsu 1959). A detailed analysis on the mechanism of mitosis through ultraviolet microbeams has been carried out by Takeda and Izutsu (1960). These earlier works were carried out exclusively in animal cells *in vivo*: somatic mitoses of newt cells or meioses of grasshopper spermatocytes.

In plant material, cells in tissue culture and pollen mother cells in *in vivo* observations are generally not suitable to study the effect of microbeams at the microscopic level. Recently a brief report has been published with regard to the disappearance of spindles and phragmoplasts after microbeam irradiations of cytoplasm by Zirkle, Uretz and Haynes (1960). They used endosperm cells of *Haemanthus Katharinae* as material. The mitoses in staminal hair cells of *Tradescantia reflexa* are also available for this purpose. However, they contain, as is well known, so many chromosomes (2n=24) with long arms that it is practically very difficult to irradiate a desirable part of individual chromosomes by microbeams and to follow their behaviour, although microbeam irradiations on polar caps and spindle poles can be available, because their development at the end of a prophase is clearly observable *in vivo*.

In this paper, the effect of UV microbeams on the mitosis of *Tradescantia* cells has been studied with regard to the development of the polar caps and the karyokinetic spindles and to their roles during the mitosis of plant cells.

Method and material

The instrument and the procedure of UV microbeam irradiation used

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in this experiment have been described in detail by Izutsu (1959) who has
designed a slightly modified instrument after the apparatus used by Uretz
and others (1954). The irradiation area of this instrument is about 2μ in
diameter. The time of irradiation for Tradescantia cells ranged from 3 to
30 minutes. This experiment was carried out at the Department of Patholo-
gy, Mie Prefectural University, School of Medicine at Tsu. The plant ma-
terial was previously transplanted from the Botanic Gardens of the University
of Tokyo to the Mie Prefectural University and cultivated.

The cells in the text-figures except Figs. 1c, 2d–e, 3c and 6c were taken
with the camera equipped with and mounted on the UV microbeam irradiation
instrument and the others with Leitz “Makam”; they were replaced after
the irradiations in moist chambers for culture. The cross-lines in the figures
indicate the position of irradiated areas. Numerals in brackets in each
figure give the time, when the cells were photographed.

The senior author expresses his cordial thanks to Professor S. Takeda,
the chair of the Department of Pathology, Mie Prefectural University, for
his kindness and generosity to use the instrument for this experiment.

Observations

1. Complete suppression of spindle development

According to developmental steps of the polar cap at the end of a pro-
phase, the UV microbeam irradiations revealed different effects on the forma-
tion of metaphase spindles. When the UV microbeam irradiations were focused
at the middle of one of the very young polar caps, the development of the
metaphase spindle was completely suppressed. The space enclosed with
double cross-lines in Fig. 1a indicates the position and area exposed to the
UV microbeams.

During the irradiation of a very young polar cap, the irradiated part
appeared pale and vacuolated later. No development of the spindle pole
was found not only at the irradiated pole side but also at the opposite side
of the cell. After the stop of the irradiation, neither formation of a meta-
phase spindle nor anaphase movement of chromosomes were found, but the
splitting of the chromosomes took place and one restitution nucleus appeared
later (Figs. 1b–c).

The final state of this nucleus was apparently similar with that induced
from the mitotic cells treated with colchicine. In the case of UV microbeam
irradiations, the movement of the chromosomes were suppressed not only
by the disintegration of the karyokinetic spindle but also by the stickiness
of the chromosomes.

2. Partial suppression of spindle development

When a developing polar cap was irradiated at a slightly later stage from
its beginning, in which the fibrillation of the nuclear content was considered
already to take place, the development of the spindle as well as the movement of the chromosomes were suppressed only at the irradiated pole side.

In Fig. 2a, the UV microbeam was focused at the middle of the upper polar cap. After 30 minutes irradiation, the exposed polar cap became indistinct diminishing its size, although the polar cytoplasmic strands remained still between the diminished spindle pole and the opposite cell wall (Fig. 2b). On the other hand, a half spindle appeared rather normally at the intact pole side (Figs. 2c–d). In spite of the suppression of the spindle development at the irradiated pole side, the splitting of the chromosomes seemed to occur normally. However, the split chromosomes belonging to the irradiated polar cap were blocked by their stickiness forming a clump of chromosomes. On the contrary, the chromosomes belonging to the intact pole showed slightly a tendency to move poleward in the half spindle, but most of them remained near the equator adhering to their partners (Fig. 2d). Then, the blocked chromosomes together with adherent ones were pushed toward the irradiated pole side by the growing of the phragmoplast which developed from the half spindle.

After the displacement of all the chromosomes toward the irradiated pole side, a cell plate appeared in the phragmoplast at the equator of the cell. Consequently, the mother cell was divided into two daughter cells: one contained all the daughter chromosomes as one clump and the other no nucleus (Fig. 2e).

In Figs. 3a–c, the UV microbeam was focused at one of the young
polar caps, which were in a slightly later step of the development than that in Fig. 2. Some of the daughter chromosomes belonging to the unirradiated spindle pole moved apparently poleward in the half spindle. However, before the chromosomes could arrive at the spindle pole, they were pushed toward the exposed pole side by the growing of the phragmoplast, which developed from the intact half spindle (Fig. 3b). In telophase, it was found that both daughter chromosome groups were forced to enter into one of the daughter cells at the irradiated pole side, which was separated from the other with a curved cell wall (Fig. 3c).
3. Nuclei before development of polar caps

Seven cells in mid- or late prophase were irradiated with the UV microbeams for 3–30 minutes (Tab. 1). After the irradiation, 3 cells in mid-prophase reversed into resting nuclei, while 4 cells in late prophase finished their mitoses in various states. Some of them showed sticky chromosome bridges in anaphase and divided into daughter nuclei unequal in size in telophase (Figs. 4a–d).

In a cell in late prophase, the microbeam was focused at one side of the equator of the nucleus. In this case, vacuole appeared at the irradiated position of the equator and many sticky chromosome bridges appeared at the opposite side of the equator in anaphase (Figs. 5b–c). This bundle of chromosome bridges hindered the further separation of the daughter chromosomes, so that a doughnut shaped nucleus appeared in telophase (Fig. 5d).

Unrelated to the position of the UV microbeam irradiations in the nuclear cavity, vacuoles appeared in the irradiated sites, and the chromosomes lying around the exposed area became sticky. Thus in plant cells, it is generally recognized that the UV microbeams induce directly vacuolization and indirectly stickiness of chromosomes and both changes at or around the irradiated area spread gradually toward the outside of the exposed areas.
4. Spindle poles in prometaphase and in later stages

In the UV microbeam irradiations on the animal cells in prometaphase or in metaphase, the investigations were centered on the aberrant chromosomes and their behaviour. In *Tradescantia* cells *in vivo*, such aberrant chromosomes are considered to occur but it is rather difficult to observe their individual morphological changes and aberrant movements, because of a large number of chromosomes and long arms crowded in the spindle.

When a spindle pole of *Tradescantia* cells in prometaphase was irradiated, some of chromosomes lagged or stuck other chromosomes or made sticky chromosome-bridges during anaphase. Therefore, the cells were separated into daughter nuclei unequal in size. The size of the daughter nuclei were determined by, whether the lagged chromosomes or those forming the chromosome bridges would join the chromosome group at the irradiated pole side or that at the intact pole side (Tab. 1). Thus the daughter nuclei at
Table 1. Changes of effects of UV microbeams on mitoses of *Tradescantia* cells in different mitotic stages

<table>
<thead>
<tr>
<th>Mitotic stages</th>
<th>Effect on mitotic figures</th>
<th>Time</th>
<th>Vacuole in nucleus</th>
<th>Reversion into resting nucleus</th>
<th>Joined daughter nuclei</th>
<th>Daughter cell without nucleus</th>
<th>Chromosome bridges</th>
<th>Daughter nuclei unequal in size</th>
<th>Normal mitoses in appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preprophase</td>
<td>C, P, P</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Prophase</td>
<td>C, P, P</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Metaphase</td>
<td>C, P, P, S</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Anaphase</td>
<td>C, P, P, S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

C, middle of equator; P, middle of polar cap or spindle pole; S, one side of equator; 1 contained large chromosomes.
the irradiated pole side were not always larger than those at the intact pole side.

As mentioned in the sections 1 and 2, the Tradescantia cells irradiated with UV microbeams in the polar cap stage showed mitotic aberrations in connection with the spindle mechanism, but the cells irradiated in other mitotic stages proceeded nearly in the normal way, although some of them showed daughter nuclei unequal in size or temporary sticky chromosome bridges in anaphase or in telophase. On the contrary to earlier mitotic stages or to mitoses in animal cells (Gaulden and Perry 1958, Takeda and Izutsu 1960), no abnormalities of the mitosis were found, when the UV microbeams were focused on the outside of the polar caps or on that of the spindle pole.

5. Vacuolization of cell contents

As already reported in the microbeam irradiation experiments on animal cells (Bloom et al 1955, Izutsu 1959, Takeda and Izutsu 1960), the vacuolization took place at the irradiated positions also in Tradescantia cells. The exposed area appeared at first as a pale spot which vacuolated later.

The nature of the vacuole contents is not clear except that the paleness at the irradiated nuclear areas is due to the decrease of DNA content (Perry 1957). The vacuolization appeared sometimes during the irradiation but sometimes a few minutes later. The spaces occupied by vacuoles increased gradually, but the increase of the vacuolated areas did not exceed, mostly a few times, the original irradiated areas.

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Figs. 6a–c. Vacuole in irradiated nucleus. a, photographed during irradiation; 5 minutes passed from the beginning of exposure. Cell in prometaphase (13:45). b, late anaphase (14:14). c, mitosis goes on normally; vacuole remains in daughter nucleus at irradiated pole side. Photographed on next day of irradiation. v: vacuole, w: cell wall.
With regard to the behaviour of the induced vacuoles, it is noteworthy that the vacuoles, once appeared in the spindle, were transported in the daughter nuclei (Figs. 6a–c), although the vacuoles lying in the phragmoplast disappeared in the cytoplasm by the disintegration of the phragmoplast. If the nuclear membrane would disappear at the end of the prophase and the spindle would remain without its own surface membrane, the maintenance of the vacuoles in the daughter nuclei may be difficult to explain as a matter of physical phenomena.

Discussion

The effects of UV microbeams on the mitotic cell were classified into two categories: one is physiological effect and the other structural effect. Both effects have been pointed out already in X-ray irradiation experiments on plant cells by Sax (1940).

The stickiness of the chromosomes and the degeneration of the spindle induced by UV microbeam irradiations are considered to belong to the physiological effects which appeared both in animal and plant cells (Bloom et al 1955, Gaulden and Perry 1958, Izutsu 1959, Takeda and Izutsu 1960). In Tradescantia cells, the stickiness is usually limited on the chromosomes belonging to the irradiated pole side or those lying near the irradiated areas.

The UV microbeam irradiations on a very young polar cap suppress wholly the development of the metaphase spindle and this fact revealed that the development of the spindle fibrils started from the polar caps. From this phenomenon, it is assumed further that the globular precursors of the spindle fibrils at the very beginning of the formation of the polar caps lost the ability to transform themselves into fibrils under the effect of the UV microbeams. All these findings concerning the formation of the karyokinetic spindle coincide with the explanation of the spindle formation based on in vivo observations by Wada (1935, 1960) and with the data on the developmental process of the spindle fibrils demonstrated in the electron micrographs by Satô (1958, 1960).

Notwithstanding that the area which is directly exposed to the UV microbeams is so limited about 2μ in diameter, the beams can suppress wholly the development of the spindle. From this phenomenon, it is concluded that the UV microbeams may induce a change of the physico-chemical condition which suppresses the fibrillation of the globular precursors and that this condition spreads to the entire cavity of the nucleus in the polar cap stage. With regard to the long distant effect of UV microbeam irradiations, Zirkle et al (1960) concluded that the effect is probably mediated by a spindle "poison" induced photochemically from a normal cytoplasmic constituent. However, in a series of analytical irradiation experiments on Tradescantia cells mentioned above, no evidence could be found to support the idea of a spindle "poison" originated from any cytoplasmic constituent.
On the contrary to the cases of the wholly suppression of the spindle development, in the partial suppression demonstrated in Figs. 2 and 3, it is concluded that the globular precursors, when irradiated, have already transformed themselves into fibrils filling the spindle cavity. In this developmental step of the spindle, two changes seem to be possible to take place as the effect of UV microbeams: one is the suppression of the formation of the chromosomal fibers and the other is the occurrence of the chromosome stickiness at the irradiated pole side. Both effects spread scarcely beyond the irradiated half spindle of the cell. Therefore, the chromosomes belonging to the intact polar cap can move poleward in the half spindle which develops later into the phragmoplast.

Another important finding is that the daughter chromosome group in the half spindle is pushed together with the other chromosome group toward the irradiated pole side by the growing of the phragmoplast. This displacement of the daughter chromosome groups has revealed that the pushing force which acts in a monopolar spindle is stronger than the pulling force induced by the shortening of the chromosomal fibers in this abnormal state of the spindle. And also the displacement of the daughter chromosome groups demonstrated in Figs. 2 and 3 may not occur, if the spindle would not be independent from the cytoplasm by the presence of its own surface membrane. A fusion of the daughter chromosomes and reconstruction of one nucleus was reported by Nakanishi (1960). He obtained such a fusion of the daughter chromosome groups by the microbeam irradiation of β-rays from the source of St$^{90}$ in the meiosis of the grasshopper spermatocytes. After microbeam irradiations on endosperm cells of Haemanthus, Zirkle et al. (1960) have found disappearance of spindles and phragmoplasts. However, their experiments lacked a confirmation whether the disappearance of achromatic figures is due to the microbeam irradiations alone or to necrobiotic changes induced by irradiations as well as other unfavourable culture conditions during experiments; the answer of this question would be given by the culture of the treated cells in the succeeding days.

Structural effects are possible to occur in the mitotic cells of Tradescantia. However, the morphology of the aberrant chromosomes and their behaviour cannot be observed exactly in vivo as those found in the grasshopper spermatocytes (Izutsu 1959, Takeda and Izutsu 1960). In Tradescantia cell, it is rather difficult to observe the shape and movement of the individual aberrant chromosomes in the anaphase, because of the large chromosome numbers and long arms crowded in the spindle. The most common and visible structural changes of the Tradescantia cells are the vacuolization of the irradiated areas. Vacuolated areas apparently increase their volumes beyond the exposed areas but the increasing remains within several times of the original areas.

Among the mitotic aberrations induced by the UV microbeam irradiations,
the following facts contribute to the elucidation of the mechanism of mitosis.

The complete suppression of the spindle development induced by the irradiation at a very young polar cap has confirmed the nuclear origin of the unit fibrils of the atractoplasm which starts from both polar caps and develops toward the equator of the nuclear cavity (Wada 1950, Satô 1960). Comparing the complete suppression with the partial suppression of the spindle development, it becomes clear that the development of the atractoplasm precedes to that of the chromosomal fibers (Wada 1950) and that a pushing force takes place by the growing of the phragmoplast, which induces the movement of daughter chromosomes which appear in a monopolar spindle in a different way from that in a normal bipolar one.

Based on the findings mentioned above, it is concluded that the globular precursors of the spindle fibrils are most sensitive and unstable for the UV microbeam irradiations among mitotic elements, and that the spindle poles must be the chemical center of the mitotic activities (Wada 1950, Wada and Satô 1958).

**Summary**

Mitotic spindles of *Tradescantia* cells were irradiated with an ultraviolet microbeam about 2 µ in diameter and its effects on the mitosis were investigated in *in vivo* observations.

1. A complete suppression of the spindle development occurs, when a polar cap is irradiated in its very young stage and a partial suppression appears when a polar cap is irradiated in its slightly later stage from its appearances.

2. In a complete suppression of the spindle development, a restitution nucleus appears, while in a partial suppression of the spindle a half spindle develops at the intact pole side, inducing blocking and sticking of the chromosomes at the irradiated pole side. When the half spindle transforms into a phragmoplast, both daughter chromosome groups are pushed by the growing of the phragmoplast to the irradiated pole side.

3. Other mitotic aberrations, such as daughter nuclei unequal in size, those with vacuoles, reversion of prophase nuclei into resting ones and occurrence of sticky chromosome bridges are obtained in cells irradiated in various mitotic stages.

4. From the results of UV microbeam irradiations on the polar caps or the spindle poles, their roles and submicroscopic structure were discussed in connection with the mechanism of mitosis.

**References**


