Some Phase Optic Observations on the Formation of a Cleavage Furrow in Beta-Irradiated Grasshopper Germ Cells

By

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(With 5 Tables)

Work hitherto on the effect of radiation on the chromosomes has mostly been done on fixed and stained materials. Recently, use being made of phase cinematography technique, valuable contributions have been made to the analysis of the mechanism of cell division under the influence of radiation and chemicals (Ohnuki 1958, Nakanishi 1959, Ohnuki and Makino 1960, Nakanishi 1960, Nakanishi, Ohnuki and Makino 1961, Nakanishi and Makino 1962). The present study deals with the formation of a cleavage furrow in grasshopper germ cells under the influence of beta-irradiation from a strontium 90 source.

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Material and method: The experiments were carried out with first spermatocytes of the grasshopper, *Podisma sapporense*. For observation, new hanging-drop preparations were made following the idea of Nakanishi (1960). The beta source was an Amersham strontium 90 plaque applicator, of which the nominal content was 20 mc (Nagai 1958). For irradiation, a spermatocyte in a suitable stage was selected and irradiated for 9 or 18 minutes so as to give approximately, 2,000 or 4,000 rads through # 1 thinness cover slip, according to the method of Nakanishi (1959). All observations were carried out at room temperature (26°–29°C) with an Olympus slidetype phase microscope with the following optics: negative high contrast objectives ×40 and ×100, in combination with a ×10 eyepiece.

Results of Observations

As controls, the formation of a cleavage furrow was serially followed in untreated spermatocytes of *Podisma sapporense* ranging from anaphase-I to

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telophase-I, with special concern for the behavior of the mitochondria. At metaphase, the mitochondria are distributed in the cytoplasm without definite orientation. At anaphase they lined up surrounding the surface of the spindle body and aggregated into a few bundles. The mitochondrial bundles showed elongation at telophase parallel to the spindle axis, stretching like bridges between the two telophase chromosome groups. Then the cleavage furrow was formed across the middle part of the mitochondrial bundles.

The duration of time required for the formation of the cleavage furrow was measured separately in the following four different stages:

Stage 1; the beginning of the formation of the cleavage furrow. Stage 2; the cleavage furrow comes into contact with the mitochondrial bundles. Stage 3; the cleavage furrow almost cuts the cell body into two halves. Stage 4; the mitochondrial bundles come together forming a single bundle. The time relation between stage 1 and 2, stage 2 and 3, and stage 3 and 4 was designated as phase-I, phase-II and phase-III, respectively. The results of observation are as shown in Table 1.

Table 1. Time relation in the formation of a cleavage furrow in grasshopper spermatocytes

Cell no.	Phase-I	Phase-II	Phase-III	Total
1	12min.	11min.	25min.	48min.
2	10	8	27	45
3	6	7	30	43
4	11	11	27	49
5	6	14	21	41
6	9	12	22	43
7	16	7	22	45
8	16	11	21	48
9	7	16	27	50
10	10	10	24	44
11	14	7	27	48
12	14	10	22	46
Average	10.9	10.3	24.6	45.8

Cells at the anaphase stage were irradiated with beta-rays at doses, of 2,000 rads or 4,000 rads. The course of the formation of the cleavage furrow in irradiated cells was generally similar to that in non-treated controls. The mitochondria in those cells exhibited nothing irregular in behavior showing an elongation into bundles, and the cleavage furrow appeared across just the middle part of the elongated mitochondrial bundles. Then the cell body was cut into two halves. After irradiation with 2,000 or 4,000 rads, the time relation of three phases mentioned above was observed. The results of observation of time relation are given in Tables 2 and 3.

Table 2. Time relation in the formation of cleavage furrow in grasshopper spermatocytes following irradiation with 2,000 rads of beta-rays.

Cell no.	Phase-I	Phase-II	Phase-III	total
1	13min.	8min.	26min.	47min.
2	10	4	27	41
3	10	12	28	50
4	19	11	13	43
5	18	13	18	49
6	11	13	19	43
7	11	13	22	46
8	9	15	22	46
Average	12.6	11.1	21.9	45.6

Table 3. Time relation in the formation of a cleavage furrow in grasshopper spermatocytes following irradiation with 4000 rads of beta-rays

Cell no.	Phase-I	Phase-II	Phase-III	total
1	4min.	13min.	30min.	47min.
2	11	12	38	61
3	8	14	28	50
4	9	9	27	45
5	4	10	38	52
6	9	17	24	50
7	10	11	25	46
8	12	6	29	47
9	13	5	30	48
Average	8.9	10.8	29.9	49.6

Mean duration of each phase of control and treated spermatocytes was calculated on the basis of the above data, and the results are shown in Tables 4 and 5. Evidence presented indicates that the mean duration of the three phases shows no particular difference between the untreated control cells and 2,000 rads

Table 4. Time relation in the formation of a cleavage furrow in controls and those treated with beta-irradiation of 2,000 rads in grasshopper spermatocytes

	Phase-I	Phase-II	Phase-III	total
Control	10.9 ± 1.03	10.3 ± 0.83	24.6 ± 0.87	45.8 ± 0.81
Treated	12.6 ± 1.35	11.1 ± 1.25	21.9 ± 1.81	45.6 ± 1.10
	$T=1.014$	$T=0.561$	$T=1.495$	$T=0.150$
	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$

irradiated ones, whereas irradiation at 4,000 rads resulted in a slight elongation of phase-III with some delay of the formation of cleavage furrow. After a statistical test the difference was proved to be $P<0.05$.

Table 5. Time relation in the formation of cleavage furrow in controls and those treated with beta-irradiation of 4,000 rads in grasshopper spermatocytes

	Phase-I	Phase-II	Phase-III	total
Control	10.9±1.03	10.3±0.82	24.6±0.87	45.8±0.81
Treated	8.9±1.06	10.8±1.27	29.9±1.68	49.6±1.61
	T=1.327	T=0.346	T=3.016	T=2.218
	P<0.05	P>0.05	P<0.01	0.01<P<0.05

Discussion

The stickiness of chromosomes after X-irradiation with comparatively higher dosages was common in the grasshopper spermatocytes (Ohnuki 1958, Ohnuki and Makino 1960). It was suggested that the stickiness of chromosomes probably induced the disturbance of the spindle mechanism. Nakanishi (1959) noted that the disturbance of anaphasic migration of the chromosomes to the poles after beta-irradiation might be caused by the stickiness of chromosomes, while the mitochondria remained unaffected. Carlson (1941, 1954) reported that large doses of X-rays applied to dividing grasshopper neuroblasts resulted in no demonstrable effect on the structure or function of the spindle. Bloom *et al.* (1955) have also observed a similar feature in cultured newt cells.

Ris (1943, 1949) reported that the chromosome-separation consisted of two separate processes; first the shortening of the chromosomal fibers which moved the chromosomes to the poles, and secondly the elongation of the spindle body which caused further separation of daughter chromosomes. Working on microdissection of dividing cells of *Chortophaga* neuroblast, Carlson (1952) concluded that the formation of a cleavage furrow is independent of the spindle and chromosomes. Makino and Nakanishi (1955) stated that cell elongation taking place in late anaphase may induce the stretching of the spindle, that the two processes go hand in hand and that they play a role in the formation of the cleavage furrow. Further, dealing with abnormal division experimentally induced with chemicals and radiation, Nakahara (1952), Makino and Nakanishi (1956), Ohnuki (1956), and Nakanishi, Ohnuki and Makino (1961) have reported that the formation of a cleavage furrow is associated with the behavior of mitochondria at least in grasshopper spermatocytes.

The results of the present experiments revealed that in cells irradiated at anaphase when the daughter chromosomes had completely separated from each other, the formation of daughter nuclei seemed to be unaffected; a cleavage furrow

was formed at the end of telophase across the middle part of the mitochondrial bundles in regular manner, though there was a slight time delay. It seems probable to the author that the delay in the formation of a cleavage furrow may be due partly, though not wholly, to a decrease of metabolic activity of cells under the influence of irradiation, but not to the damage of certain cellular elements, since the behavior of the spindle and mitochondria seemed not to be disturbed.

Summary

The present study deals with the formation of a cleavage furrow in living grasshopper spermatocytes which had received beta-irradiation at 2,000 rads and 4,000 rads.

The results of the present experiments revealed that in cells irradiated at anaphase when the daughter chromosomes had completely separated from each other, the formation of daughter nuclei seemed to be unaffected; a cleavage furrow was formed at the end of telophase across the middle part of the mitochondrial bundles in regular manner, though there was a slight time delay.

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