ELIMINATION OF X-RAY INDUCED ABERRANT CHROMOSOMES IN THE ROOT MERISTEMS OF TWO-ROWED BARLEY¹⁾

TAKAJI IKUSHIMA AND SADA0 ICHIKAWA

Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Kyoto

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In our studies of radiation-induced somatic mutations in diploid oats heterozygous for the albino gene, *al*, it was found that elimination of somatic mutant (albino) cells occurred during plant growth (Nishiyama *et al.* 1964, 1966, Ichikawa and Ikushima 1967). Also, it was concluded that the somatic mutations in this material often were induced through two-hit events, probably such as interstitial deletion of the chromosome segment carrying the normal gene (Nishiyama *et al.* 1964, 1966). Similar conclusions have been made by Sparrow and Cuany (1959), Sparrow *et al.* (1961), Matsumura *et al.* (1963) and others in several additional plant species. Amano and Smith (1965) confirmed that many of the X-ray induced mutations in corn were due to deletions. Based on these findings and conclusions, it is supposed that such an elimination of mutant cells in somatic tissues might be related to the occurrence of the deletion-type somatic mutations mentioned above. Loss of chromosome segments generally has been thought to be the main cause of death or loss of reproductive ability of individual cells by radiations. However, there has been little evidence presented which definitely prove that chromosomal losses play the main role in producing such radiation damage. Therefore, it is necessary to confirm whether or not the cells having chromosomal aberrations can survive through successive mitotic division cycles after irradiation, and how such chromosomal aberrations contribute to the depression of growth.

The present paper describes the mitotic behavior of cells carrying chromosomal aberrations in X-rayed barley root tips.

MATERIALS AND METHODS

Seeds of a cultivated variety, Schwanhals, of two-rowed barley (*Hordeum distichum* L., $2n=14$) were used. The seeds were soaked for 24 hrs in the dark at $20\pm 1^\circ\text{C}$ prior to irradiation. These seeds were then treated with a single moderate exposure, 300 R, of X rays delivered at an exposure rate of 354 R/min. This exposure was selected to induce many chromosomal breaks without causing delayed or abnormal mitotic divisions. Our preliminary examination showed that exposures higher than 300 R frequently induced a delay and abnormalities (predominantly stickiness of chromosomes) of mitosis in the same material. All the X-irradiations were performed in the light with a Philips' X-ray

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machine operated at 200kV and 19mA, using 1.0mm Al filter. During irradiation the seed embryos were oriented upward in a single layer. The seeds were put back in the dark immediately after irradiation and permitted to continue the germination at the same temperature. Primary root tips were fixed with ethanol-glacial acetic acid (3:1) at six-hour intervals from 30 to 132 hrs after soaking. Cytological preparations of the root tips (1.5mm long) were made after staining with Feulgen's leuco-basic fuchsin, one root tip to a slide. Various types of chromosomal aberrations were scored at anaphase and metaphase. About 1000 to 1200 cells at anaphase were carefully observed at each fixation time, and 150 metaphase cells at every other fixation time. For the metaphase observations, root tips were treated with 0.05 per cent colchicine solution for two hours prior to fixation. The mitotic index also was determined at every fixation time from 5000 meristematic cells.

RESULTS AND DISCUSSION

Mitotic indices obtained from both irradiated (300R) and control root tips are presented in Table 1. The data show that there is no significant difference in the mitotic indices between the irradiated and non-irradiated root-tip meristems. However, the indices are somewhat lower in the irradiated than in the non-irradiated root tips at the earliest two fixation times, 30 and 36 hrs after soaking, suggesting a slightly delayed onset of mitosis caused by the X-ray treatment. The indices obtained from the irradiated root tips are plotted against time after soaking in Fig. 1. The curve drawn in this figure suggests that the meristematic cells in the root tips pass through, on the average, three division cycles within 132 hrs after soaking. The three peaks of the mitotic indices appear at 42, 78 and 120 to 126 hrs after soaking, but the third peak is not clear probably because of a reduced synchronism after the second cycle of mitosis. Thus, a fair assessment of the cell cycle time is about 36 hrs in this material (see Fig. 1).

The chromosomal aberrations observed were mostly of the chromosome type, agreeing well with our earlier observation (Ichikawa *et al.* 1965). The frequencies of dicentric

Table 1. Mitotic indices in X-rayed (300R) and non-irradiated root-tip meristems determined 30 to 132 hrs after soaking

Time after soaking (hr)	Mitotic index (%)		Time after soaking (hr)	Mitotic index (%)	
	300 R	0 R		300 R	0 R
30	3.3	3.8	84	14.9	14.5
36	17.5	19.3	90	11.0	—
42	19.7	19.8	96	9.8	9.7
48	15.8	16.0	102	10.1	—
54	15.6	15.4	108	10.5	10.3
60	10.6	10.7	114	11.0	—
66	7.9	7.9	120	11.7	11.7
72	11.8	11.8	126	12.1	—
78	18.0	—	132	10.8	10.3

Fig. 1. Mitotic index vs. time after soaking in the barley root-tip meristems irradiated with 300 R of X rays. Two horizontal arrows indicate the average cell cycle time estimated from the mitotic indices.

chromosome bridges and fragments observed at anaphase are presented in Table 2. No anaphase scoring was carried out at 30 and 36 hrs after soaking, because anaphase figures were observed infrequently at these times. The frequencies of the dicentric bridges and fragments decrease rather rapidly as time increases. The relationship of the bridge frequency vs. time after soaking is shown in Fig. 2. It is very clear from this figure that the number of dicentric bridges per 100 anaphase cells decreases rapidly with increasing time, *i. e.*, with the progression of cell generation. The frequencies observed 84 and 120 hrs after soaking are about 27 and 7 per cent of that observed at 48 hrs, respectively. These times, 48, 84 and 120 hrs after soaking, are the times when anaphase cells at the first, second and third mitotic divisions, respectively, can be observed more frequently than at any other time.

If the two centromeres of a dicentric chromosome move to the poles independently of each other, one can expect that half of the dicentric chromosomes will form bridges at anaphase. Indeed, Conger (1965) observed that 47 per cent of the dicentrics in *Tradescantia* microspores failed to form bridges at anaphase. The dicentrics whose two centromeres moved to the same pole thus forming no bridge will be transmitted into the next cell generation, while those which formed bridges will not be passed on as dicentrics unless they undergo a breakage-fusion-bridge cycle. McClintock (1941) reported that the breakage-fusion-bridge cycle continues through successive cell generations in corn gametophytes and endosperms but not in the embryos and sporophytes. Therefore, it seems reasonable to expect that the frequency of the anaphase dicentric bridges is halved every cell generation. That is, the frequencies of the dicentric bridges at the second and third cell divisions are expected to be a half and a quarter of that at the first division. The observed frequencies at the second and third divisions are obviously lower than the expectations (Fig. 2).

The frequencies of dicentrics and rings observed at metaphase also showed similar

Table 2. Frequencies of chromosomal aberrations at anaphase in the barley root-tip meristems irradiated with 300 R of X rays

Time after soaking (hr)	No. anaphase cells observed	Dicentric bridges*		Fragments	
		no.	per 100 cells	no.	per 100 cells
Irradiated					
42	1218	167	13.7	182	14.9
48	1034	146	14.1	162	15.7
54	1056	109	10.3	178	16.9
60	1079	89	8.2	110	10.1
66	1196	71	5.9	—	—
72	1138	71	6.2	65	5.7
78	1151	66	5.7	59	5.1
84	1070	42	3.9	45	4.2
90	1106	42	3.8	—	—
96	1051	24	2.3	30	2.9
108	999	10	1.0	—	—
120	1029	10	1.0	2	0.7
132	1136	14	1.2	—	—
Control					
48	1057	3	0.3	0	0.0

* Those of the chromosome type were scored as two bridges and those of the chromatid type as one. Almost all of the bridges observed were of the former type.

Fig. 2. Frequency of dicentric bridges at anaphase vs. time after soaking in the barley root-tip meristems irradiated with 300 R of X rays. The central and right-side horizontal lines indicate the expected frequencies of dicentric bridges at the second and third mitotic divisions, *i. e.*, a half and a quarter of the frequency at the first division (the left-side horizontal line), respectively, expectations based on an assumption that 50 per cent of dicentric chromosomes form bridges at anaphase (see text).

Table 3. Frequencies of chromosomal aberrations at metaphase in the barley root-tip meristems irradiated with 300 R of X rays

Time after soaking (hr)	No. metaphase cells observed	Dicentrics		Rings		Deletions*		
		no.	per 100 cells	no.	per 100 cells	no.	per 100 cells	
Irradiated								
36	150	17	11.3	6	4.0	28	18.7	
48	150	14	9.3	4	2.7	30	20.0	
60	150	5	3.3	2	1.3	17	11.3	
72	150	5	3.3	1	0.7	11	7.3	
84	150	4	2.7	2	1.3	9	6.0	
96	150	2	1.3	1	0.7	8	5.3	
120	150	0	0.0	0	0.0	5	3.3	
Control								
48	150	0	0.0	0	0.0	0	0.0	

* The chromosomes with detectable deletions.

rapid decreases (Table 3). The frequencies of the dicentrics presented in this table are apparently lower than those at anaphase shown in Table 2. The lower frequencies at metaphase are due mainly to our scoring method, *i. e.*, at anaphase a "one" chromosome-type bridge was scored as two (see the footnote of Table 2), while all the dicentrics detected at metaphase were of the chromosome type and were scored as one. However, the frequencies at metaphase are still lower than the expected result of twice the anaphase value (*i. e.*, they should be nearly equal to each other by our scoring method), an assumption based on the random movement of the two centromeres of the dicentrics mentioned above (compare 11.3 per 100 metaphase cells with a half of 13.7 per 100 anaphase cells at 36 and 42 hrs after soaking, respectively). Thus the lower frequencies may also be connected with the following: For the metaphase observations, only the cells whose chromosomes were well spread and separated from each other were scored. Therefore, it seems highly probable that the cells having dicentric chromosomes might have been excluded from the samples more frequently than those with no aberrations or those with only simple interchanges. A non-random movement of the two centromeres of the dicentric chromosomes is another possibility which cannot be ruled out. Namely, if more than 50 per cent of the dicentrics formed bridges at anaphase, a similar result could be obtained. This latter possibility seems, however, rather remote when Conger's (1965) results and the inaccuracy of the scorings at metaphase are taken into consideration. Thus the main cause of the discrepancy between the frequencies at metaphase and those at anaphase is considered to be the difficulty of accurately scoring the dicentrics at metaphase.

The frequency of acentric fragments observed at anaphase decreased from cycle to cycle of mitosis at about the same rate as dicentric bridges (Table 2), although the correlation of frequencies does not seem so high because of the very possibly excluded (from our scoring) fragments hidden in the chromosome masses near the poles. On the other hand, a similar but somewhat slower decreasing rate was found for the

chromosomes having obvious deletions detected at metaphase (Table 3). A rapid decrease of various chromosomal aberrations during successive mitotic cycles in plant meristems has been observed by other workers (Thoday 1951, Wakonig-Vaartaja and Read 1962, Clowes 1963, Evans 1965, Grant 1965).

The present results indicate that a considerable amount, possibly more than 50 per cent, of the cells carrying dicentric chromosomes are eliminated from the meristematic cell population. This faster rate of decrease than expected for dicentric bridges (Fig. 2) seems to be attributable to an inferior reproductive ability or loss of reproductive integrity of the cells carrying induced dicentric chromosomes. The occurrence of any dicentric chromosome is inevitably accompanied with eventual loss of chromosome segments. Radiation-induced loss of reproductive integrity of cells has been generally attributed to cell death or mitotic arrest ("permanent interphase", see Van't Hof and Sparrow 1963) probably as the result of genic or chromosomal loss or unbalance caused by chromosome aberrations, or to a premature differentiation (see Dewey and Howard 1963, Evans 1965). Evans (1965) stated that chromosomal aberration might not merit the importance usually ascribed. However, the present study suggests that loss of chromosome segments must be one of the important factors leading to loss of reproductive integrity. The question that to what extent such loss of reproductive integrity contributes to the depression of growth in multi-cellular meristems is the next problem to be solved. Although many evidences recently accumulated demonstrate the direct correlation between loss of reproductive integrity and growth inhibition in a single-meristematic-cell system, *Tradescantia* stamen hairs (Davies 1963, Alvarez and Sparrow 1965, Nayar and Sparrow 1967, Ichikawa and Sparrow 1967a, 1967b, 1967c, Ichikawa *et al.* 1967), little such evidence has been obtained from multi-cellular meristems.

SUMMARY

Frequencies of chromosomal aberrations were examined during three successive cell generations in the root meristem of two-rowed barley following X-ray irradiation of 24-hr soaked seeds with a moderate exposure of 300 R. Dicentric bridges and acentric fragments observed at anaphase and dicentrics, rings and chromosomes with obvious deletions detected at metaphase were scored. Frequencies of these chromosomal aberrations decreased rapidly with the progress of cell generation. Their rapid reduction in number, especially of the dicentric bridges which decreased much more rapidly than expected, indicates that elimination of the cells carrying aberrant chromosomes occurs in the meristematic cell population. The elimination seems to be attributable to the loss of reproductive integrity of the cells carrying such aberrations, probably as a result of the loss of chromosome segments.

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