FAST NEUTRON RADIATION AND LOCALISED CHROMOSOME BREAKAGE

M. S. SWAMINATHAN AND A. T. NATARAJAN

Indian Agricultural Research Institute, New Delhi

IT is of interest while studying the cytological effects of radiations to determine whether breaks occur at random in the chromosomes, or whether certain points are specially liable to be broken. Such studies can be carried out only in favourable material where certain chromosomes can be marked and their division cycle separately followed. Ford *et al.*[1] have recently used chromosome markers in the study of the problem of regeneration of particular groups of cells in mice. The present paper deals with certain observations on the effects of fast neutron radiation on mitosis in root tip cells of *Triticum monococcum* (var. Japanese Early) using a pair of satellited chromosomes as markers.

Dry seeds of *T. monococcum* were irradiated for 3 hours with fast neutrons of source strength 10^9 neutrons/cm.^{2}/sec. (approx.) over a 2π solid angle from the cascade generator of the Tata Institute of Fundamental Research, Bombay, using the reaction $d(Be^9B^{10})n$. The seeds were germinated in petri dishes at 24° C. and the effects of the treatment on somatic chromosome division were studied in Feulgen root tip squashes of material fixed in acetic alcohol (1 : 3) without any pre-treatment 24, 72 and 96 hours after germination. Preparations from untreated *T. monococcum* seeds germinated and fixed in a similar way, served as controls.

The control preparations showed the normal division figures characteristic of *T. monococcum*. In the material treated with neutrons, cell division proceeded without inhibition. At prophase and metaphase of the dividing nuclei, many chromosome breaks and interchanges were seen. Other cytological aberrations included the presence of bridges and fragments at anaphase and micronuclei at telophase and formation of occasional polyploid nuclei. Some cells showed clumping at metaphase and bridges at anaphase due to a surface stickiness caused by the deposition of nucleic acid on chromosomes in a fluid unpolymerized state.

FIG. 1. A normal somatic complement of *T. monococcum* ($2n = 14$). Arrows point to Sat. II pair which have been used as markers in this study. FIG. 2. Neutron treated root tip cell showing 3 Sat. chromosomes intact (→) and one Sat. II chromosome broken near the supernumerary constriction region in the short arm (↛). (Magnification of photographs, × 1800.)

In the normal chromosome complement of *T. monococcum*, there are two pairs of satellite-bearing chromosomes, one of which is somewhat longer than the other (designated Sat-chromosome I and II ; relative lengths 12·6 μ and 10·4 μ respectively). Among the remaining five pairs of chromosomes, the centromere is submedian in position in 4 pairs and median in the other. In the short arm of the satellited chromosomes, there is a weak supernumerary constriction. These chromosomes are readily recognisable in well-spread plates (Fig. 1) and hence were used as markers in the following study.

Only chromosome breaks occurred at metaphase and no chromatid breaks could be identified. The frequency of occurrence of chromosome breakages was analysed in 315 metaphase plates; in 210 of these, no breaks were seen and in the rest the number of breaks per cell varied from 1 to 20. The relative frequency with which cells containing different numbers of breaks were found, follows Poisson distribution thus indicating that the formation of a chromosome break is unaffected by the presence or absence of other breaks in the cell.

In preparations made from material fixed 24 hours after germination, a critical study of the types and points of origin of breaks could be done in 64 clear metaphase plates. The analysis showed that a break frequently occurred near the supernumerary constriction region of one chromosome belonging to the Sat. II pair (Fig. 2). The frequency of occurrence of this particular break and the total number of breaks observed in different cells are listed in Table I. Breaks in the Sat. I pair were noticed in 7 cells. Only in two cells which had 20 chromosome breaks, both the Sat. II chromosomes were affected in the same region. Some breaks also occurred in the distal part of the Sat. II chromosomes. None of the other fragments, however, showed the recurrence characteristic of the proximal break in one of the Sat. II chromosomes.

TABLE I

Description	No. of cells	No. of localised breaks in Sat. II	Total No. of breaks
Regular	11	0	0
One chromosome break	20	9	20
Two chromosome breaks	21	12	42
Three do	5	2	15
Four do	2	1	8
Five do	2	1	10
Six do	1	1	6
Twenty do	2	4	40
TOTAL ..	64	30	141

The total chromosome length in a somatic complement of *T. monococcum* was calculated to be 172·2 μ, of which the length of the Sat. II pair was 20·8 μ. If chromosome breakages following neutron radiation are caused completely at random, the chances for any one of the observed breaks to occur in the Sat. II chromosomes will be proportional to their length in the complement. On this basis, approximately one in every 8·3 breaks could occur in the Sat. II chromosomes. The observed figure of 30 out of a total of 141 breaks does not, however, fit with this expectation ($\chi^2 = 11{\cdot}30$; $P < 0{\cdot}01$). This would suggest that the region near the supernumerary constriction of Sat. II chromosomes is preferentially disposed to breakage by neutron radiation. There was no evidence in preparations made 24, 72 and 96 hours after germination of the occurrence of a general restitution, and hence the results may not be attributable to non-random reunion and restitution of broken fragments instead of to non-random breakage. It is not possible to determine by microscopical observations whether the breakage always occurs only in one particular chromosome of the Sat. II pair. The occurrence of a single Sat. II break in most of the cells suggests that some degree of differentiation with reference to localised neutron sensitivity may occur in the apparently homologous pair of chromosomes. Also, if the particular break in a Sat. II chromosome occurs at random among the pair, a break in both the chromosomes can be expected to occur in 25% of the cells which show a Sat. II break. Only two among the 28 cells with Sat. II breaks showed breakage in both the chromosomes (P between 0·05 and 0·02). Thus, localised breakage appears to occur in only one chromosome of the pair and not at random in either of them.

From the results so far reported in literature, it appears that breaks produced by radiations both between different chromosomes and along the same chromosome are generally distributed at random. The position is, however, quite different with regard to chemical mutagens. Observations by Ford[2] and others in *Vicia faba* have clearly shown that the heterochromatic regions of chromosomes are more susceptible to damage by radiomimetic chemicals like Nitrogen Mustard than the euchromatic parts. There are indications that the heterochromatic regions of chromosomes may also be more sensitive to breakage induced by radiation with X-rays, ultraviolet rays and gamma rays.[3,4]

In both slow and fast neutron irradiated material, chromosome breaks have usually been assumed to occur at random. Konzak and Singleton[5] have, however, very recently found that different endosperm factors in chromosome 9 of maize respond quite differently to thermal neutron radiation, thus indicating some basic difference in the breakability of chromosomal regions. The results of the present study provide for the first time cytologi-

cal evidence for the existence of a non-random sensitivity of chromosome segments to fast neutron action. Examination of chromosomes in root tip cells subjected to cold treatment did not reveal the presence of any prominent heterochromatic segment in the region where breakage frequently occurs. In case the non-random breakage is confined to only one of the Sat. II chromosome pair, as seems likely from our data, it may suggest the presence of a submicroscopic differentiation between the homologous pair of chromosomes at the vulnerable region. In a similar study carried out in the same variety of *T. monococcum* using β radiation from ^{32}P, we found no evidence for a preferential susceptibility to breakage of any segment in either Sat. II or the other chromosomes. Thus, it would appear that the localised breakage caused by fast neutron radiation is probably a correlated consequence of the specific properties of the neutron particles and the concerned chromosome segment.

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