LOCALIZED CHROMOSOME BREAKAGE INDUCED BY MITOMYCIN C IN TRADESCANTIA PALUDOSA AND VICIA FABA ROOT TIPS

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Received December 4, 1970

Various patterns of the non-random and mutagen-specific distribution of chromosome aberrations have been established in plants and mammals (Evans 1962; Kihlman 1966). Even after treatment with ionizing radiations, the aberrations are not completely randomly distributed between and within chromosomes. The nonrandomness, however, is much more remarkable after treatment with chemical agents.

In the studies with higher plants, most of the available information concerning the distribution of chemically induced chromosome aberrations has been obtained with root-tip cells of Vicia faba (e.g., McLeish 1953; Kihlman 1963; Natarajan and Upadhya 1964). Since many chemical agents affect mainly heterochromatic regions or secondary constrictions, we must consider the specificity of breakage for localized segments as a reflection of the property of the chromosome itself as well as that of the agents. The localization of chromosome breaks which is specific to a mutagen may be a useful tool in determining chromosome structure. It is particularly true for the case of the agents whose chemical actions are well delineated. Additional knowledge concerning the effects of other compounds manifesting the specificity for different chromosomal regions may lend insight into the structure of these regions.

Mitomycin C (MC) is shown to inhibit DNA synthesis and degrade cellular DNA in bacteria (Shiba et al. 1959; Sekiguchi and Takagi 1960) and mammalian cells (Reich et al. 1961; Shatkin et al. 1962), but does not arrest the synthesis of RNA or protein (Reich and Tatum 1960; Kuroda and Furuyama 1963). Recent evidence indicates that MC may act as an alkylating agent (Schwartz et al. 1963) which links complementary DNA strands together by the formation of covalent bonds (Iyer and Szybalski 1963). MC has been reported on several occasions to cause mitotic inhibition as well as chromosome breakage. The chromosome aberrations induced by MC were also localized in heterochromatic regions in Vicia (Merz 1961) and secondary constrictions in cultured cells of human leucocytes (Cohen and Shaw 1964; Nowell 1964).

In the present paper, an attempt to compare the chromosomes of two different plants for sensitivity to MC was undertaken, using root-tip cells of Vicia faba in which there are some heterochromatic regions on the chromosomes and those of Tradescantia paludosa lacking visible heterochromatin.

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MATERIALS AND METHODS

The materials used in the present experiments were the primary roots of *Vicia faba* and the adventitious roots from sprigs of *Tradescantia paludosa*. The treatment with mitomycin C were carried out at 20±1°C in the dark when roots were about 7 cm long. The same temperature was used during the recovery period. The roots of both plants were treated for one hour with mitomycin solution of 10 μg per ml which was freshly prepared with distilled water. After treatment the roots were washed well with running tap water, and transferred in the aerated tap water and allowed to recover for 24 to 72 hours. Cytological preparations were made as Feulgen squashes after 3 hour’s pretreatment with 0.05% colchicine and fixation in acetic acid-alcohol (1 : 3). In Figures and Tables, the fixation times are expressed as the times from the end of treatment. The observations were carried out with cells at metaphase.

RESULTS

(a) Types and frequencies of chromosome aberrations

In both *Tradescantia* and *Vicia*, almost all the structural changes scored with metaphase cells were of the chromatid-type, *i.e.*, isochromatid breaks with sister-reunion (SR), isochromatid gaps (G’’), chromatid breaks (B’), chromatid gaps (G’), chromatid interchanges (T’’), and triradials (Tri). However, triradials were of infrequent occurrence. In both of the treated materials, a few chromosome-type aberrations were observed, but their frequency is almost consistent with that in untreated roots. Therefore, the data with chromosome-type aberrations were omitted.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Recovery time (hr)</th>
<th>Number of cells observed</th>
<th>Abnormal cells (%)</th>
<th>Frequency of aberrations</th>
<th>Aberrations per 100 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tradescantia</em></td>
<td>24</td>
<td>400</td>
<td>14</td>
<td>G’’ (3.0) SR (54.5) B’ (18.1) G’ (13.6) T’’ (10.6) Tri (0)</td>
<td>16.5 (100)</td>
</tr>
<tr>
<td><em>paludosa</em></td>
<td>48</td>
<td>400</td>
<td>9</td>
<td>G’’ (8.9) SR (62.2) B’ (6.7) G’ (6.7) T’’ (15.6) Tri (2.2)</td>
<td>11.3 (100)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>400</td>
<td>4</td>
<td>G’’ (4.8) SR (85.7) B’ (0) G’ (9.5) T’’ (0) Tri (0)</td>
<td>5.3 (100)</td>
</tr>
<tr>
<td><em>Vicia faba</em></td>
<td>24</td>
<td>400</td>
<td>31</td>
<td>G’’ (21.3) SR (59.8) B’ (5.3) G’ (4.1) T’’ (8.9) Tri (0)</td>
<td>42.3 (100)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>300</td>
<td>23</td>
<td>G’’ (21.3) SR (29.3) B’ (18.7) G’ (2.7) T’’ (28.0) Tri (0)</td>
<td>25.0 (100)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>9</td>
<td>G’’ (42.1) SR (52.6) B’ (0) G’ (5.3) T’’ (0) Tri (0)</td>
<td>9.5 (100)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages of aberrations (for 100 aberrations).
The types and frequencies of chromatid-type aberrations at various times of recovery are presented in Table 1. The data on the preparations fixed at the times of 24 or 48 hours' recovery show that all types of aberrations listed in Table 1, except Tri, occurred. In both plants, the isochromatid breaks of SR were higher in their frequency than those of any other types of aberrations. This trend in the frequency of the isochromatid breaks hold true for all the fixation times.

It can be deduced from Table 1 that the frequency of each type of aberrations varies with the recovery time. However, the mode of the change with the time of recovery differed among the various types of aberrations and also between the plants. In *Tradescantia*, the relative frequency of SR's increases with increasing time between treatment and fixation, whereas that of T'' remains at a rather constant value or slightly decreases. In the case of *Vicia*, the relative frequency of SR's once decreases, i.e., at the 48 hours, and again increases. On the contrary, the relative value of T'' reaches a maximum value after the recovery of 48 hours and finally falls off.

The efficiency of the agent in inducing aberrations, which is expressed as aberrations per 100 cells in the table, was sharply different between both materials. The yields of aberrations were higher in *Vicia* than *Tradescantia* at all the recovery times shown in the table. This phenomenon suggests the difference in the susceptibility of chromosomes to MC between the two plants. However, if the cells with which aberrations were scored were not those at the first division after MC-treatment, the comparison for the susceptibility is impossible. Although the durations of various mitotic parameters of *Tradescantia* cells have been determined by Wimber and Quastler (1963), as well as those of *Vicia* cells, the use of these data is not permitted if mitotic delays were caused by the MC-treatment. Considerable numbers of T'', B', and G' were observed at 24 or 48 hours in both plants, thus suggesting that the cells observed were those at the first metaphase after treatment. In the case of *Tradescantia*, however, the large proportions of the aberrations scored at 48 or 72 hours were SR's. Therefore, there is a suspicion that these cells with SR's were the second mitotic cells. A fact that mitotic delays were caused in *Tradescantia* cells, as well as in *Vicia* cells, was shown in the experiments of the continuous colchicine treatment during the whole periods of recovery for 24, 48 or 72 hours (Table 2). Since the cells divided in the presence of colchicine should be tetraploid (at the time of observation), the rare occurrence

<table>
<thead>
<tr>
<th>Recovery time (hr)</th>
<th>Number of metaphases observed</th>
<th>Diploid</th>
<th>Tetraploids</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>230</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>200</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>200</td>
<td>197</td>
<td>3</td>
</tr>
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</table>
of tetraploid cells even after 72 hours of the recovery indicates that the duration of a cell cycle was delayed to a great extent by the MC-treatment and the cells with SR’s, observed at 48 or 72 hours, are the first mitotic cells.

(b) Distribution of breaks between and within chromosomes

The chromosome complement of *Vicia faba* consists of five pairs of chromosomes with subterminal centromere (S-chromosomes) of nearly equal length and one pair of those with a median centromere and a nucleolar constriction in one arm (M-chromosomes). The methodology used here for identifying the chromosomes and the heterochromatic regions is similar to that described by Natarajan and Upadhya (1964), i.e., M, Sa, Sb (three pairs distinguished as the pairs having Sb1, Sb2, or Sb3 segments of heterochromatin) and Sc.

<table>
<thead>
<tr>
<th>Types of breaks</th>
<th>Numbers of breaks</th>
<th>S/M ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-chromosomes</td>
<td>M-chromosomes</td>
</tr>
<tr>
<td>Isochromatid breaks</td>
<td>162</td>
<td>38</td>
</tr>
<tr>
<td>Chromatid breaks</td>
<td>72</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>50</td>
</tr>
</tbody>
</table>


The differential susceptibility to MC between M- and S-chromosomes is indicated in Table 3. When breaks are distributed in somewhat random fashion, the theoretical ratio (S/M ratio) based on the relative lengths of the chromosomes at mitosis is approximately 2.5 (cf. Kihlman 1957). In the present experiment, the S/M ratio was 4.3 for isochromatid breaks and 6.0 for chromatid breaks, thus indicating the favorness for S-chromosomes.

The relative frequency of breaks at the various points of chromosomes is summarized in Fig. 1. Each frequency is represented as a percent of total breaks from many chromosomes. The distribution of chromosome aberrations was found to be non-random. In the two chromosome groups of M and Sb, breakages did occur in seven distinct segments. Five of these correspond to the heterochromatic regions of Sb1, Sb2, Sb3, M1, and M2 which are clearly visible after a cold treatment of the plants. Other localization-points are also specific to the certain regions, i.e., the centromeres of Sb-chromosomes, and nucleolar constrictions of M-chromosomes. Highly localized breaks were found in the heterochromatic regions of Sb1 and Sb2. These regions are the large heterochromatic segments in *Vicia*. This can be a reason why the highest frequency of breaks is found to occur in these regions. There were a few breaks at the nucleolar constrictions, as mentioned above, but they were not taken as those specific to this treatment, because these regions were found to be prone to breakage
even in the control preparations to some extent. It is significant that no break involving any other regions of *Vicia* chromosomes occurred, at least as far as the conditions used in the present study is concerned. A centromeric region of M-chromosome appears to be comparatively invulnerable to MC at the dose of MC used in the present study.

The mitotic chromosomes of *Tradescantia paludosa* consist of almost submetacentric chromosomes. Since these chromosomes are nearly the same in length and have no visible segments of heterochromatin and secondary constriction, it is impossible to know the differential susceptibility to MC between the chromosomes. Therefore, all the submetacentric chromosomes were subdivided into seven regions as shown in Fig. 2, and the relative frequency of breaks at these regions was recorded. The data are presented in Fig. 2. A pattern different from that obtained with *Vicia* chromosomes emerged, when one examined the regional sensitivity of the chromosomes following MC-treatment with respect to isochromatid breaks or chromatid breaks. The most striking effect of MC to *Tradescantia* chromosomes is that the proximal and distal regions of long arms are favored for breakage, unlike the case with *Vicia* chromosomes.
DISCUSSION

The results reported here show that in both cells of Vicia and Tradescantia MC induces chromatid-type aberrations, so far as the metaphase cells at the first mitosis after treatment were concerned. Thus it is apparent that in Tradescantia cells MC is capable to produce the breakage regardless of the absence of visible heterochromatin. However, the frequency of breaks observed after exposure to the same concentration of MC and the same duration of the treatment was higher in Vicia than Tradescantia. The difference between the two plants in susceptibility of their chromosomes to MC might be due to the presence or absence of visible heterochromatin. To indicate clearly this difference in susceptibility, it is required to compare the two plants with the frequencies that were obtained with cells fixed after the more shorter periods of recovery. In other series of experiments (unpublished data) in which the fixation of roots was carried out at the 3, 6, 9, and 12 hours of recovery in Tradescantia and at the 6, 11, and 19 hours in Vicia after the identical MC-treatment for 15 min, the highest yields of aberrations in Tradescantia were obtained with the 3 and 6 hours samples, whereas the maximum yield in Vicia was observed with the 19 hours sample. However, the maximum yield in Tradescantia was about one-half of that of Vicia. Therefore, it can be concluded that the chromosomes of Vicia are more susceptible to
MC than those of *Tradescantia*, though the possibility of the difference with penetration of MC into their cells is not excluded. Recently Natarajan and Ahnström (1969) investigated the chromosome breaking effects of some chemicals and gamma rays on the root meristematic cells of *Nigella damascena* lacking any detectable heterochromatin, and found that all the agents tested produce relatively fewer aberrations, as compared to *Vicia faba* which are similar in their total chromosome lengths to *Nigella* cells. The observed difference between *Vicia* and *Nigella* was interpreted as due to the presence or absence of heterochromatin in their chromosome complements.

The distributions of breaks observed in the present study are not random between or within chromosomes in *Vicia* and also within chromosomes in the case of *Tradescantia*. The breaks were localized to several regions in their chromosome complements, in either case. Similar localization has been observed for MC by Merz (1961) and Rao and Natarajan (1967) in *Vicia*, as well as by Cohen and Shaw (1964) and Nowell (1964) in human leucocyte cultures. According to Rao and Natarajan (1967), MC-induced breaks were maximum in their frequency in the Sbl region and their results are in agreement with the data of the present study.

It is well known in plant and animal cells that the distribution of chemically induced aberrations is not random between and within chromosomes (Evans 1962; Kihlman 1966). Various suggestions have been made for explaining this nonrandomness, such as the differential distribution of the chemical agent in question inside nuclei (Kihlman 1963) or the organization of chromosomes in interphase nuclei (Rao and Natarajan 1967). Other factors such as the reaction mechanism of chemical agents, as well as the chemical composition and physiological activity of the affected chromosome regions, may be also involved in the production of localized chromosome breakage.

Using mammalian cells in culture, Somers and Hsu (1962) have demonstrated the localization of breaks induced by 5-bromodeoxyuridine or hydroxylamine in identifiable chromosomal segments and correlated these regions as being rich in adenine-thymine (A-T) pairs, or guanine-cytosine (G-C) pairs in the DNA of these chromosomal segments. Natarajan and Upadhya (1964) have undertaken a similar study of chromosome breakage in *Vicia* induced by ethyl-methane-sulfonate (which attacks G) and hydroxylamine (which attacks C) to determine the localization points of the chromosome breakage and to see whether the two agents induce the preferential breakage due to the differences in the base composition of DNA of these regions. The points of breakage induced by these two chemicals have been found to be extremely overlapping and all the breaks induced were exclusively confined to the certain points, *e.g.*, centromeres and Sbl, Sb2, and Sb3 regions of the Sb-chromosomes. Many sites impaired by MC which were shown in the present study overlap with those impaired by ethyl-methane-sulfonate or hydroxylamine. Thus the localization of MC-induced breaks at these sites would suggest that the specificity of the attack is due to the selective action of MC on G-C rich regions of chromosomes. Caspersson *et al.* (1968), using *Vicia* chromosomes, observed a specific binding of the fluorescent quinacrine mustard to the sites corresponding to the heterochromatic segments which were defined by a cold treatment of the plant, and supposed that these segments may be the loci with a particulary high guanine content. Sbl and Sb2 regions are the largest heterochromatic segments in
*Vicia.* Therefore, MC-induced aberrations would be localized in these regions to a large extent.

On the one hand, Lann (1955) was unable to see any localization of aberrations induced by di-epoxypropyl ether in *Tradescantia paludosa* (see review by Loveless, 1966). The distribution of the breaks induced by this agent differs from the localized-type distribution of MC-induced breaks which was shown in the present study using *Tradescantia paludosa.* This localization of MC-induced breaks in *Tradescantia* chromosomes might be a reflection of the presence of G-C rich segments in the chromosomes which do not become detectable as visible heterochromatic segments by the cold treatment of the plants. The results of the biochemical studies on the mechanism of MC action lend some support to our presumption. Matsumoto and Lark (1964) suggested that the formation of cross links between the complementary strands of DNA molecules is the mechanism whereby MC inhibits DNA synthesis. There is evidence suggesting that the formation of the bonds of this type is favored by a high content of G-C paris and then these bases may be the site of MC action (Szybalski and Iyer 1964).

The last question in this discussion refers to the difference with regard to the distribution of breaks between our results and those of Merz (1961), who indicated some specificity of breakage for the M-chromosomes and also indicated that approximately 80 percent of the isochromatid breaks in the M-chromosomes were situated at the nucleolar constrictions in his experiments using lateral root tips of *Vicia.* However, the differences in experimental conditions may account for partly the discrepancies. These discrepancies might be explained in either of the two ways: (1) A difference between the Merz’s experiment and our own is in his use of lateral roots and our use of primary roots. In a limited series of experiments using *Vicia,* however, Rieger and Michaelis (1961) showed that there was no difference in the number or type of aberrations produced by Myleran or nitrogen mustard, whether the materials used were lateral or main roots, so long as these were of the same length (about 2 cm). Rao and Natarajan (1967), using lateral roots of *Vicia,* showed that most of MC-induced aberrations were localized to the known heterochromatic regions of Sb-chromosomes, in particular in the Sb1 region, while in a few cases nucleolar constrictions were also involved. Their results are in agreement with those presented here. These findings do not permit to accept this explanation. (2) The distribution of MC-induced breaks varies with recovery times after treatment. The data of Merz were obtained from the observation at 72 hours after removal of MC, while those reported here were obtained from the observation at 24 hours. Kihlman (1963) noted in *Vicia* roots treated with 8-ethoxycaffeine that the isochromatid breaks were localized to the nucleolar constrictions in M-chromosomes and the degree of localization increased with increasing time between treatment and fixation, *i.e.*, the most extreme localization was observed in cells treated in early interphase, in which 90 percent or more of the aberrations were concentrated in the nucleolar constrictions. Grant and Heslot (1966) have shown that, from the changes of the S/M ratio, the distribution of the aberrations induced by nitroso-ethyl-urethane and nitroso-methyl-urethane between and within chromosomes changes with the stage of DNA synthetic period of treated cells and suggested that the change of S/M ratios might result from the fact that the rates of DNA synthesis in different heterochro-
matic segments varies with time, because not all the heterochromatic ones show the asynchrony of labeling by tritium thymidine. Thus they suggested that the changes of the aberration pattern with time, together with the dose-dependent toxic delay, might resolve some of the differences that were said to exist between different radiomimetic chemicals. This alternative seems to be accepted as an explanation to solve the discrepancy between Merz's data and ours obtained using the different roots of *Vicia*.

Thus one may conclude that many factors such as the reaction mechanism of the agent in question and its distribution in the nucleus, as well as the chemical composition and physiological activity of the affected chromosomal regions, are involved in the production of localized chromosome breakage.

**SUMMARY**

1. The pattern and distribution of chromosome aberrations induced by mitomycin C were compared between *Vicia faba* which possesses heterochromatic regions and *Tradescantia paludosa* lacking visible heterochromatin in all the chromosomes, using meristematic cells of their roots.

2. Mitomycin C produced chromatid-type aberrations in both *Vicia* and *Tradescantia*. The frequency of aberrations was higher in *Vicia* than *Tradescantia*. The distribution of aberrations was found to be nonrandom with a marked excess of breaks in some heterochromatic regions of Sb-chromosomes in *Vicia* and at some segments of submetacentric chromosomes in *Tradescantia*.

3. The mechanism of action of mitomycin C and the possible reasons for these localized effects were discussed.

**ACKNOWLEDGMENTS**

The author wishes to thank heartily to Prof. Emer. Dr. H. Matsuura and Prof. Dr. I. Harada for their kind guidance and encouragement during this work. Thanks are also due to Assist. Prof. Dr. S. Tanifuji for his advices and suggestions given during this work.

**LITERATURE CITED**


