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Position-specific effects in the mutagenic action of mitomycin C on the chromosomes of *Hordeum vulgare* L.

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Summary. The mutagenic action of mitomycin C (MMC) on the chromosomes of two reconstructed karyotypes of barley was studied. MMC-induced chromatid aberrations were found to be distributed non-randomly along the chromosomes. The regions situated next to the secondary constrictions of chromosomes 6 and 7 appeared to be clearly pronounced aberration "hot spots". In these segments, intercalary deletions and duplication-deletions were the most frequently induced aberration types. The comparative analysis of the frequency and localization of MMC-induced aberrations in the chromosomes of the two karyotype variants, which differ from each other by the position of the "hot spot" segments, provided new evidence about the influence of the segment transposition on the "hot spot" expressivity. The most remarkable finding obtained in the study is that the size of the segment involved in both intercalary deletions and duplication-deletions proved to be strongly affected by the structural peculiarity of the reconstructed chromosome. The possible reasons underlying this finding are discussed.

Key words: Chromosome aberrations – Translocation karyotypes – Giemsa banding – Mitomycin C – *Hordeum vulgare*

Introduction

The specific distribution of chemically induced chromosome rearrangements has been established for a number of different organisms, including plants, animals and man (Kihlman 1966; Loveless 1966). More recently this problem has received much attention in the studies of Rieger and coworkers (Rieger and Michaelis 1972; Rieger et al. 1977; Schubert et al. 1979, 1985). The conclusion to be drawn from their experiments is that, depending on the nature of the mutagen and the structure of the karyotype used, the frequency and the spectrum (type and localization) of the structural mutations may vary in a rather large range.

In view of the importance of these findings, further detailed investigations in other organisms are necessary as well. Barley (*Hordeum vulgare* L.), because of its convenience for cytogenetic studies, may even open new possibilities for investigating both theoretical and applied aspects of the regional specificity of the mutagens. This paper deals with the mutagenic action of mitomycin C on the chromosomes of two reconstructed barley karyotypes which differ from each other by the chromosomal position of the most pronounced "hot spot" segments.

Materials and methods

Plant materials

The materials used were germinating seeds (resting seeds were pre-soaked for 15 min in tap water and afterwards kept on moist filter paper for 18 h in petri dishes) of two reconstructed karyotypes – T-1586 and T-21. The structural details of these karyotypes are given in Fig. 1. Karyotype T-1586 was produced by gamma-irradiation of the standard variety Freya and involves a single reciprocal translocation between chromosomes 3 and 4 (Gecheff 1978). Karyotype T-21 (Gecheff, unpublished results) is identical to T-1586 with respect to translocation 3–4 and contains an additional reciprocal translocation between chromosomes 6 and 7.

Mutagenic treatment and cytological procedures

For mutagenic treatment, germinating seeds were immersed in 0.0008% aqueous solution of MMC (Sigma) for 2 h. After treatment with the mutagen, the seeds were rinsed intensively for 10 min and germinated on moist filter paper in petri dishes until fixation of the primary roots (six fixations at different times covering the first mitotic cycle after treatment, indicated in Fig. 2 as "recovery time"). Before fixation (1:3 glacial acetic acid and ethanol), the roots were immersed for 2 h in a solution of

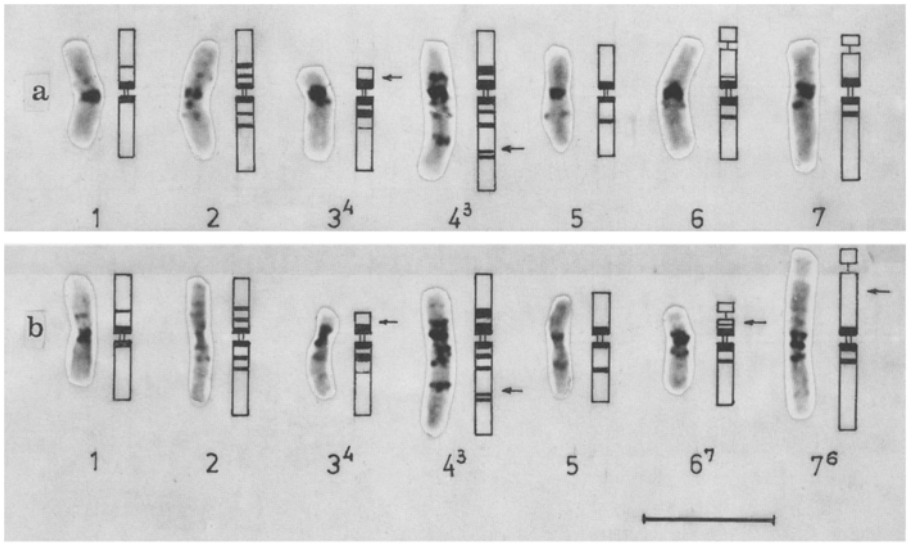

Fig. 1 a and b. Idiograms of Giemsa-banded chromosomes of barley-reconstructed karyotypes **a** T-1586 and **b** T-21. *Arrows* indicate the putative translocation break points. *Bar* represents 10 μm

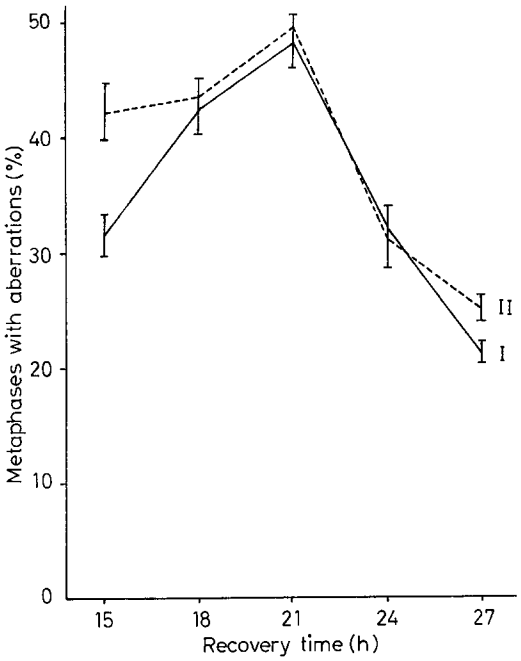

Fig. 2. The frequencies of aberrations at different recovery times after treatment of karyotypes T-1586 (I) and T-21 (II) with MMC (0.0008%, 2 h)

0.03% colchicine saturated with α-bromonaphtalene. After maceration of the roots in 4% pectinase (12 min), permanent Feulgen squashes were prepared.

For differential staining of somatic metaphase chromosomes, a slightly modified Giemsa-(N)-banding technique of Singh and Tsuchiya (1982) was used.

All treatments were carried out in dark chamber at 24 °C.

Scoring procedures

Scoring of aberrations was done in the first metaphase after application of the mutagen. For chromosomal mapping of MMC-induced aberrations, the method developed by Rieger and Michaelis (1972) for *Vicia faba* was applied. The metaphase chromosomes, which are easily interdistinguishable in both T-1586 and T-21, were subdivided into 53 segments of nearly equal sizes (Figs. 3 and 4). The centromeres and secondary constrictions were designated as individual segments. The segments were numbered respective to their position in the standard karyotype. The total number of all aberrations induced in each karyotype was taken as 100 and the percent involvement of the individual chromosome segment in isochromatid breaks, intercalary deletions, duplication-deletions and translocations was determined. Aberrations located in chromosome 1, which is practically metacentric, were distributed equally on both arms.

All experiments were carried out in at least three replications. The data were analysed statistically according to the formula of Rieger et al. (1975).

Results

The frequencies of MMC-induced aberrations after mutagenic treatments of both T-1586 and T-21 are shown in Fig. 2. There is no significant difference in the sensitivity of the karyotype variants tested; nearly the same yield of aberrations is obtained at recovery time 21 h, when the highest effects were scored. The differences which appear at 15 h and 27 h after treatment are probably due to the karyotype-specific effects of MMC on cell cycle progression. Also, no essential differences were observed in respect to the types of aberrations induced; in both T-1586 and T-21 almost all structural mutations were of chromatid type (isochromatid breaks, intercalary deletions, duplication-deletions, chromatid translocations and a few chromatid breaks).

Fig. 3 shows the non-random pattern of intrachromosomal distribution of MMC-induced aberration break points in karyotype T-1586. Segment 47 of chro-

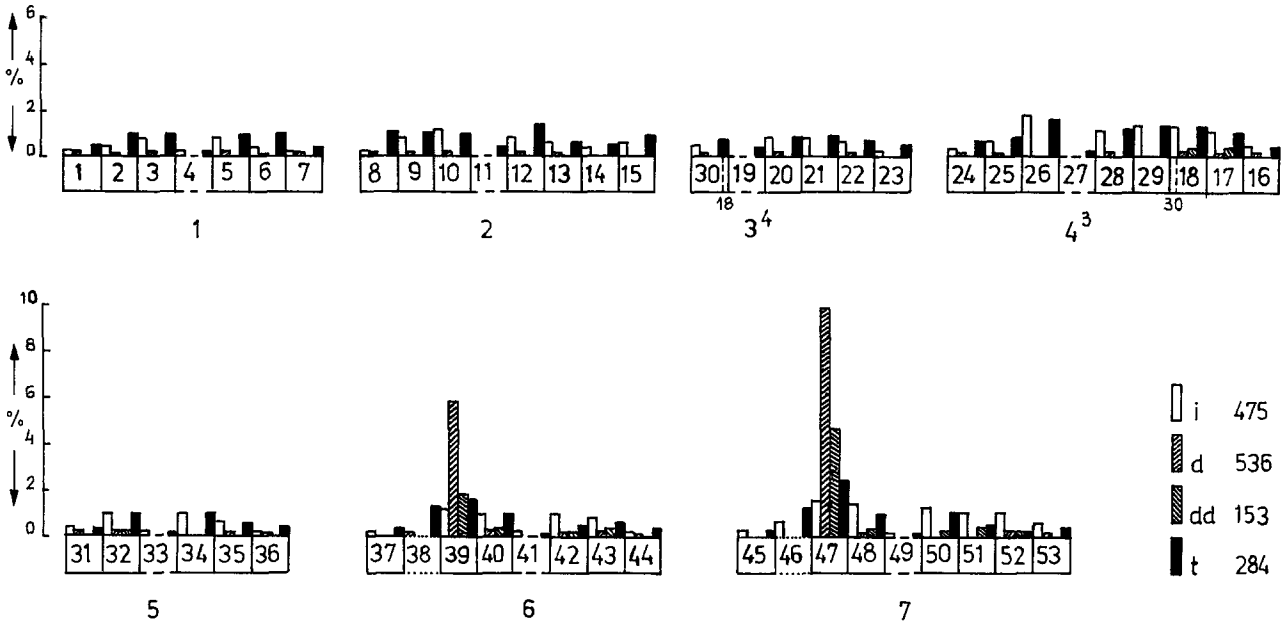

Fig. 3. The distribution of MMC-induced chromatid aberrations (i = isochromatid breaks, d = intercalary deletions, dd = duplication-deletions, t = chromatid translocations) along the chromosomes of karyotype T-1586

mosome 7 and segment 39 of chromosme 6 appear to be the most pronounced aberration "hot spots". This seems to be true for all types of aberrations but it is mostly expressed for duplication-deletions and especially for intercalary deletions (minutes). Moreover, the involvement of segment 47 in these aberration types appears to be higher. Thus, about 43% of the intercalary deletions (122 out of 284) and about 35% of the duplication-deletions (54 out of 153) were found to be localized at this segment. At the same time, the percentage involvements of segment 39 in these aberration types are 29% and 16%, respectively. A characteristic feature of these aberrations is that in neraly all cases, very small regions situated next to the secondary constrictions in segments 39 and 47 are involved (Fig. 5a–d). It should further be noted in Fig. 3 that some other segments, namely segments 9, 10 and 12 of chromosome 2; 26, 28, 29 and 18 of chromosome 4^3; 32 and 34 of chromosome 5; 40 and 42 of chromosome 6, and 48 and 50 of chromosome 7 are also found to represent statistically significant aberration "hot spots". However, nearly all of these segments which contain heterochromatin as defined by the presence of Giemsa bands (Fig. 1a) show a very low expressivity of their aberration "hot spots", i.e. their involvement in aberrations (mainly isolocus breaks and chromatid translocations) just surpasses the upper confidence limit (1% level) for random distribution.

The pattern of intrachromosomal distribution of MMC-induced aberrations in karyotype T-21 is presented in Fig. 4. The only reason to use T-21 in this study was that the standard position of the most sensitive segments 39 and 47 is specifically changed; owing to reciprocal translocation between chromosomes 6 and 7, segment 39 (together with some other segments) is transposed to the short arm of chromosome 7, so that in the newly reconstructed chromosome 7^6 the two segments become arranged tandemly (Fig. 4). This position of the segments was established by the length measurements of the metaphase chromosomes and differential staining with Giemsa (Fig. 1b). It may be seen in Fig. 4 that after treatment of T-21, segments 39 and 47 keep their high sensitivity to the mutagenic action of MMC. It becomes, also evident from these data that intercalary deletions and duplication-deletions again appear to be the most frequent aberration types in these segments. However, while the relative involvement in aberrations of segment 47 remains the same in both karyotypes (about 14%–15%), the involvement in aberrations of segment 39 in the new position (karyotype T-21) is significantly increased (about 30% of all aberrations induced versus 10% in the case of T-1586). It should be noted, in addition, that the different reaction of segment 39 in this case is obviously due to the increased predilection of this region for formation of intercalary deletions (more than three times compared to T-1586) and duplication-deletions (about four times).

The most remarkable result obtained in this study is that the alteration of the chromosome constitution (due to reconstruction) may exert a clear-cut influence on the size of the chromosome segment which is involved in MMC-induced chromatid intrachanges. In Fig. 5 are shown some representative types of the most frequently

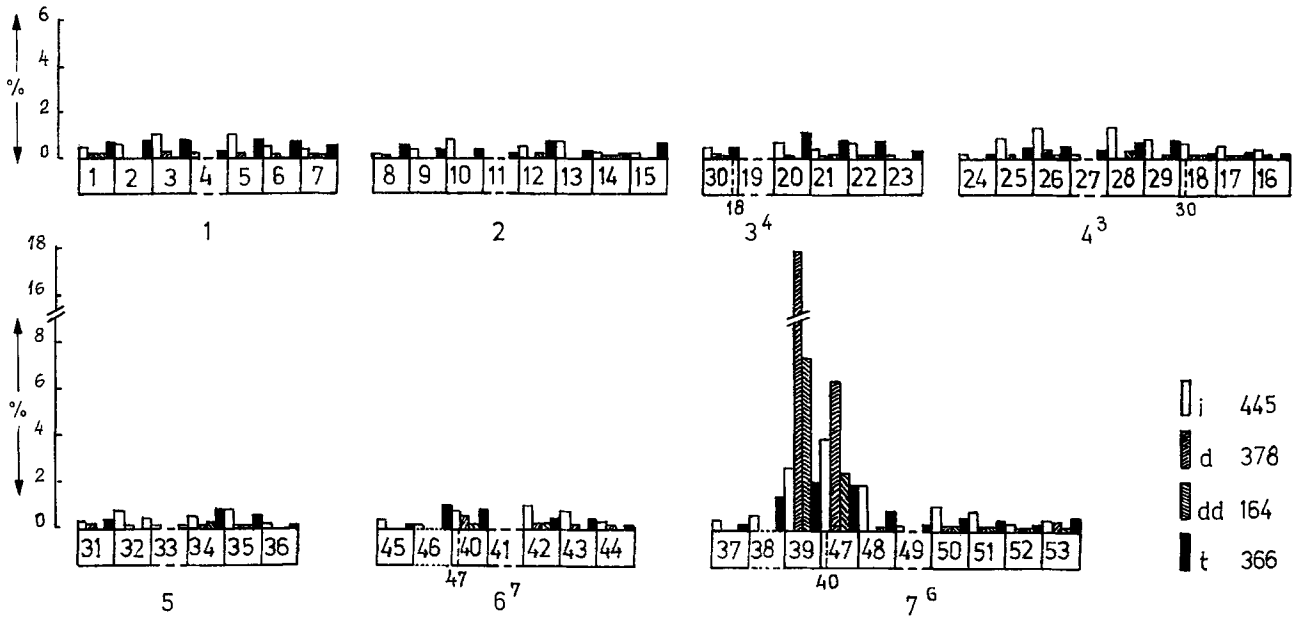

Fig. 4. The distribution of MMC-induced chromatid aberrations (i = isochromatid breaks, d = intercalary deletions, dd = duplication-deletions, t = chromatid translocations) along the chromosomes of karyotype T-21

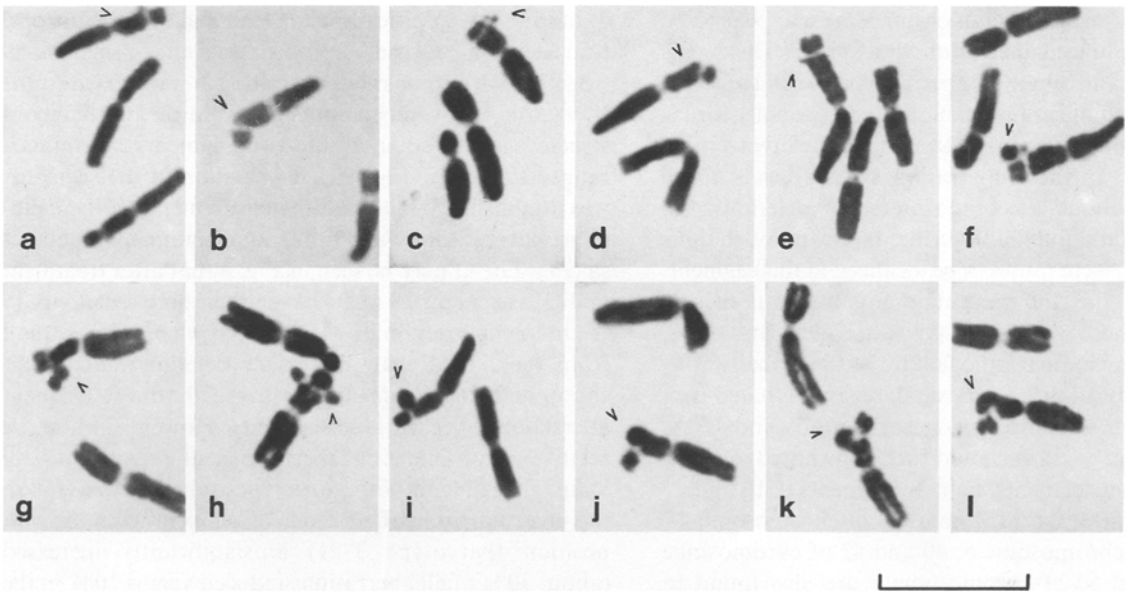

Fig. 5a–l. Representative types of MMC-induced chromatid intrachanges. Aberrations induced in T-1586: **a** intercalary deletion and **b** duplication-deletion in segment 39; **c** intercalary deletion and **d** duplication-deletion in segment 47. Aberrations induced in T-21: **e–i** intercalary deletions and **j–l** duplication-deletions in segment 39, showing significant differences in the size of the region involved in the intrachanges. *Bar* represents 10 μm

induced aberrations in both T-1586 and T-21. It can be seen that in the case of T-21 (unlike T-1586), the size of the region involved in intercalary deletions (Fig. 5e–i) and duplication-deletions (Fig. 5j–l) belonging to segment 39 varies in a wide range. What is remarkable is that the majority of these aberrations, when induced in this region of karyotype T-21, involve the whole segment 39 or exceed its size involving a part of the next segment 47 (Fig. 5i–l). Moreover, the intercalary deletions localized in segment 39 are sometimes expressed as unilateral (Fig. 5g) or bilateral (Fig. 5h) double fragments, affecting both chromatids at homologous loci.

Discussion

Summarizing the data in this study, three main conclusions can be drawn: (1) MMC-induced chromatid aberrations were found to be distributed non-randomly along the chromosomes of barley; (2) there was clearly pronounced position effect in the expressivity of the induced aberration "hot spots"; (3) the size of the chromosome segment involved in both intercalary deletions and duplication-deletions proved to be strongly affected by the structural peculiarity of the chromosome.

The localized chromosome breakage of MMC has been repeatedly investigated in *V. faba* (Merz 1961; Rao and Natarajan 1967; Utsumi 1971; Rieger et al. 1975; Schubert et al. 1984) and human cells (Nowell 1964; Cohen and Shaw 1964; Brogger and Johansen 1972; Morad et al. 1973). In both organisms, the distribution of aberration break points along the chromosomes has been characterized by a preferential involvement of heterochromatin-containing regions. In addition, the secondary constrictions of chromosomes have been found to be the most pronounced aberration "hot spots".

This study is a first attempt in barley to provide more detailed data about the correlation of the distribution pattern of MMC-induced aberrations, on the one hand, and the chromosome location of Giemsa bands (as indicative of the presence of heterochromatin), on the other. In general, our results are in accordance with the conclusion of the studies mentioned above that the potential aberration "hot spots" are usually situated in chromosome regions containing heterochromatin. This is especially true for segments showing preferential involvement in isolocus breaks and chromatid translocations. Compared to the studies with some alkylating agents, however, such as ethyleneimine (Gecheff 1978) and ethylmethanesulphonate (Gecheff 1983), which were carried out under the same experimental conditions, the MMC capacity of inducing structural rearrangements in the centromere regions and other chromosome segments known as heterochromatic proved to be considerably lower. Since MMC is known to act as an alkylating agent (Schwartz et al. 1963), such difference indicates that reasons other than the nature of the primary lesions induced in DNA by the mutagens are probably responsible for the character of the aberration distribution patterns.

An important point to be discussed in this study is the behaviour of segments 39 and 47, which appeared to be the most pronounced aberration "hot spots" in both karyotypes. These segments, occupying analogous regions in the satellite arms of chromosomes 6 and 7, seem to be structurally identical. In these experiments, we fail to reveal Giemsa bands in these regions. This fact, however, should not be accepted as evidence of the absence of heterochromatin, since by means of similar technique (Georgiev et al. 1985), clear-cut Gimesa bands were established in both segments. In the context of this study, the observations of Morad et al. (1973) and Brogger (1974) in human karyotype are also of interest. They have established a frequent induction by MMC of "lateral protrusion of one chromatid" or "laterally extended chromatin" in the secondary constrictions of chromosomes 1, 9 and 16. According to the authors, the natrue of these structural changes is not known, but it seems quite probable that they might have the same origin as MMC-induced chromatid intrachanges in the analogous regions of barley chromosomes (segments 39 and 47), which were so frequently observed in this study. The phenomenon of preferential involvement of specific types of aberrations in particular chromosome regions, originally established in *V. faba* (Rieger et al. 1977) and barley (Nicoloff et al. 1979) is probably indicative of the existence of essential differences in the mechanisms of induction of different aberration types and/or may be due to the structural differences between different aberration "hot spot" segments.

The comparative analysis of the frequency and localization of chromosome aberrations induced by MMC in segments 39 and 47 of the karyotypes used provided new evidence about the influence of segment transposition and structural features of reconstructed chromosomes on the "hot spot" expressivity. For the present, this problem is mostly developed in *V. faba* (Rieger and Michaelis 1972; Schubert et al. 1979). Based on the great amount of experimental data, a working hypothesis has been proposed (Schubert et al. 1985) that potential "hot spot" segment can be maximally expressed when only one occurs per chromosome, while any combination of two or more such segments on the same chromosome should result in a reduced expressivity of one of them. This study allows us to verify the validity of the hypothesis under experimental conditions which have not been tested in *V. faba*, as the two "hot spot" segments of karyotype T-21 are situated tandemly in the satellite arm of chromosome 7^6. No reduction in the expressivity of the aberration "hot spots" of this chromosome was observed. Instead, the involvement of one of them (segment 39) in intercalary deletions and duplication-deletions was significantly increased. Whatever the causes of this specific behaviour of the most sensitive segments in barley, it becomes evident that our results do not support the hypothesis mentioned above.

The last question in this discussion refers to the most interesting finding of the study that the specific constitution of chromosome 7^6 in the majority of the cases resulted in increasing the size of the region involved in MMC-induced intercalary deletions and duplication-deletions in segment 39. What might be the possible reasons underlying this finding? The breakage-and-reunion hypothesis and exchange hypothesis are still the most widely accept-

ed mechanisms explaining the formation of chromosome aberrations. Although in the production of the terminal chromatid deletions, the breakage-and-reunion mechanism was found to prevail (Heddle and Bodycote 1970), it seems most unlikely that the same holds true for intercalary deletions and duplication-deletions. The exchange hypothesis (Revell 1959), in which chromatid aberrations are considered to be complete or incomplete exchanges that occur in the neck of little loops in the chromosomes, seems to be the most reliable pathway leading to the production of these two types of aberrations. If so, the size of the segment involved in chromatid intrachanges will depend on the size of these loops.

As far as clearly pronounced differences in the involvement of segments in intercalary deletions and duplication-deletions were observed, it might further be assumed that the sites of loop formation are non-randomly distributed along the interphase chromosomes and are structurally determined. Based upon these assumptions, the alteration in the size of the regions involved in MMC-induced intercalary deletions and duplication-deletions in segment 39 may be explained with the rearrangement during chromosome reconstruction of the sites in which the formation of loops may occur. This explanation seems rather probable also in the view of our preliminary data with other mutagens (experiments in progress) showing that this reaction of segment 39 is independent of the nature of the mutagens used.

References

Brogger A (1974) Different pattern of chromosome exchanges induced by methyl-methanesulphonate and mitomycin C in human cells. Hereditas 77:205–208

Brogger A, Johansen J (1972) A model for production of chromosome damage by mitomycin C. Chromosoma 38:95–104

Cohen MM, Shaw MW (1964) Effects of mitomycin C on human chromosomes. J Cell Biol 23:386–395

Gecheff KI (1978) Mutagenic interaction of caffeine on ethylene imine-induced chromosomal damage in a reconstructed karyotype of barley. Mutat Res 50:77–83

Gecheff KI (1983) About the influence of caffeine on the repair of chemically induced gene and structural mutations in *Hordeum vulgare*. Genet Plant Breed 16:265–273

Georgiev S, Gecheff K, Nicoloff H, Künzel G, Rieger R (1985) Giemsa banding as a means of identification of reconstructed chromosomes in barley. Biol Zentralbl 104:29–34

Heddle JA, Bodycote DJ (1970) On the formation of chromosomal aberrations. Mutat Res 9:117–126

Kihlman BA (1966) Action of chemicals on dividing cell. Prentice-Hall, Englewood Cliffs/NJ

Loveless A (1966) Genetic and allied effects of alkylating agents. Butterworths, London

Merz T (1961) Effect of mitomycin C on lateral root-tip chromosomes of *Vicia faba*. Science 133:329–330

Morad M, Jonasson J, Lindsten J (1973) Distribution of mitomycin C induced breaks on human chromosomes. Hereditas 74:273–282

Nicoloff H, Rieger R, Michaelis A (1979) Deletion clustering in specific chromosome segments of *Hordeum vulgare* and *Vicia faba*. Biol Zentralbl 98:527–535

Nowell PC (1964) Mitotic inhibition and chromosome damage by mitomycin in human leukocyte cultures. Exp Cell Res 33:445–449

Rao RN, Natarajan AT (1967) Somatic association in relation to chemically induced chromosome aberrations in *Vicia faba*. Genetics 57:821–835

Revell SH (1959) The accurate estimation of chromatid breakage, and its relevance to a new interpretation of chromatid aberrations induced by ionizing radiations. Proc R Soc London Ser B 150:563–589

Rieger R, Michaelis A (1972) Effects of chromosome repatterning in *Vicia faba*. 1. Aberration distribution, aberration spectrum, and karyotype sensitivity after treatment with ethanol of differently reconstructed chromosome complements. Biol Zentralbl 91:151–169

Rieger R, Michaelis A, Schubert I, Döbel P, Jank W (1975) Non-random intrachromosomal distribution of chromatid aberrations induced by X-rays, alkylating agents and ethanol in *Vicia faba*. Mutat Res 27:69–79

Rieger R, Michaelis A, Schubert I, Kaina B (1977) Effects of chromosome repatterning in *Vicia faba*. 2. Aberration clustering after treatment with chemical mutagens and X-rays as affected by segment transposition. Biol Zentralbl 96:161–182

Schubert I, Rieger R, Michaelis A (1979) Effects of chromosome repatterning in *Vicia faba*. 3. On the influence of segment transposition on differential "mutagen sensitivity" of *Vicia faba* chromosomes. Biol Zentralbl 98:13–20

Schubert I, Hubner C, Rieger R, Michaelis A (1984) Effects of chromosome repatterning in *Vicia faba*. 4. Translocation clustering in the NOR as affected by its chromosomal position. Biol Zentralbl 103:529–542

Schubert I, Rieger R, Michaelis A (1985) Effects of chromosome repatterning in *Vicia faba*. 7. The influence of hot spot duplication on frequency and chromosomal distribution of maleic hydrazide-induced chromatid aberrations. Biol Zentralbl 104:403–409

Schwartz HS, Sodergren EE, Philips FS (1963) Mitomycin C: Chemical and biological studies on alkylation. Science 142:1181–1183

Singh RJ, Tsuchiya T (1982) An improved Giemsa-N-banding technique for the identification of barley chromosomes. J Hered 73:227–229

Utsumi S (1971) Localized chromosome breakage induced by mitomycin C in *Tradescantia paludosa* and *Vicia faba* root tips. Jpn J Genet 46:125–134