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Mutations in rDNA. 2. Effects of Actinomycin D on Chromatid Aberration Induction in Nucleolus Organizer Regions

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Summary. A reconstructed karyotype of barley with all chromosomes interdistinguishable was treated with hydroxyurea (HU) and Actinomycin D (Act D). The distribution pattern of chromatid aberrations after treatment with HU alone is characterized by a marked preferential involvement in chromatid translocations of segments 36 (NOR of satellite chromosome 6) and 43 (NOR of satellite chromosome 7). Act D applied at the low concentration of 0.05 μg/ml (4.10^{-8} M) before HU treatment, or combined with HU, was found to cause an apparent decrease of HU-induced aberration frequencies in NORs. The exchanges in both segments proved to be approximately a half lower after Act D application when compared to the respective controls (treatment with HU alone). A recovery period of 5 h between the prolonged pretreatment with Act D (15 h) and the HU treatment eliminated the effect of the drug. The possible dependence of mutation induction upon the transcriptional activity of rDNA in NORs after Act D application is discussed.

Key words: Chromatid aberrations – NOR – Transcription – Actinomycin D – Reconstructed karyotype

Introduction

Since the finding was made that Act D inhibits DNA dependent RNA synthesis without preventing DNA replication (Kirk 1960; Reich et al. 1962; Reich 1964), the effects of this antibiotic have been studied in many different experiments. It has been reported Act D to cause cartain chromosome changes (Isawa et al. 1963; Beerman 1965; Ostertag and Kersten 1965; Berendes 1968; Miles 1970), to promote recombination frequency (Suzuki 1963, 1965; Sinha and Helgason 1969), to suppress the expression of some recessive mutations, i.e. to induce phenotypic reversions (Burdette 1961; Mathan 1967; Fisher 1967; Sinha and Helgason 1969), to induce mutations (Fisher et al. 1975) or in some cases to act as an antimutagen (Mukherjec 1965; Puglisi 1968).

Changes in the ultrastructure of cells after treatment with Act D have also been observed. In most of them the nucleolus was found to be initially involved upon prolonged treatment with this antibiotic (Journey and Goldstein 1961; Bal 1970). These changes have invariably been connected with the preferential binding of Act D to high GC content DNA, which is localized in the nucleolus (Birnstiel et al. 1968).

When applied at higher concentrations Act D is a non-specific inhibitor of all species of chromosomal RNA, while at low concentration of 0.04 μg/ml it is shown to be a highly selective inhibitor of rRNA transcription (Perry 1962, 1963; Perry and Kelley 1970).

Based on these findings it might be expected that Act D at low concentrations would open up some possibility of studying the relation, if any, between mutation induction and transcriptional activity of rDNA which is localized in NORs and which is known to be the site of rRNA transcription. This assumption is plausible when one considers the experimental data which indicates dependence of chromatid aberration induction upon transcriptional activity of rDNA in NORs of different translocation lines in barley (Nicoloff 1981).

In this paper we report on a clear dependence of the chromosome mutation induction in NORs upon application of Act D at a low concentration in barley.

Materials and Methods

A reconstructed karyotype MK 14/2034 of barley has been treated with HU and Act D. This karyotype originated by means of reciprocal translocations which involved chromosome 1 and chromosome 7 of the standard complement of var. 'Elgina' and chromosome 3 and 4 of the standard complement

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of var. 'Frigga' (Künzel and Nicoloff 1979). All seven chromosome pairs are morphologically distinguishable. In order to localize the aberrations, the karyotype was subdivided into 48 segments (Künzel and Nicoloff 1979; Nicoloff 1981) whose individual involvement in aberrations was tested. Four types of induced chromatid aberrations were scored and located for each segment: isolocus breaks (i), duplication-deletions (dd), intercalary deletions (d) and chromatid translocations (t). The total number of these aberrations was taken as 100% and the percentage involvement of the individual segments in these aberration types respectively presented. Confidence limits (α–1%) were calculated for the segments. For analyzing the differential response of the segments after Act D application, the method of comparing two parameters of the binomial model φ_1 and φ_2, representing controlled and treated variants, was used (Brownlle 1975).

Presoaked seeds (1 h in tap water) kept on moist filter paper for 19 h in an aerated desiccator (24 °C) were treated with HU (6 h, $3 \cdot 10^{-3}$ M, 24 °C) and Act D ($4 \cdot 10^{-6}$ M, 6–15–19 h, 24 °C). The solutions of HU and Act D were freshly prepared for each treatment. The treatment with Act D was administrated: as pretreatment (19 h) immediately before the mutagen treatment; as a simultaneous treatment (6 h) with the mutagen and as pretreatment for 15 h followed by a subsequent Act D – free recovery period of 5 h before the HU treatment. Parallel controls (treatments with HU alone) were scored for each Act D treatment respectively. After treatment seeds were germinated in Petri dishes (24 °C) and fixed (1:3 glacial acetic acid and ethanol) after periods (recovery times) of 12, 18, 21, 24 and 27 h. Before fixation, the germinated seeds were immersed (2 h) in a solution of 0.025% colchicine saturated with α-bromonaphthalene. Following maceration of the root tips in pectinase (4%, 30 min) permanent Feulgen squashes were prepared.

Results

The pattern of intrachromosomal distribution of chromatid aberrations induced after HU treatment of the karyotype MK 14/2034 is shown in Figs. 1, 3 and 5. As was also observed in previous studies (Nicoloff 1981) the distribution pattern of chromatid aberrations is characterized by a marked preferential involvement in chromatid translocations of segments 36 (NOR of chromosome 6) and 43 (NOR of chromosome 7). The clustering of chromatid translocations in segment 43 tends to be invariably higher than that found in segment 36. The frequencies of aberrations in both segments surpassed by three- to fivefold the upper limit of confidence intervals (36). Therefore segments 36 and 43 represent "hot spots" of agent-specific aberration localization when HU as a mutagen is used (for review see Nicoloff 1981).

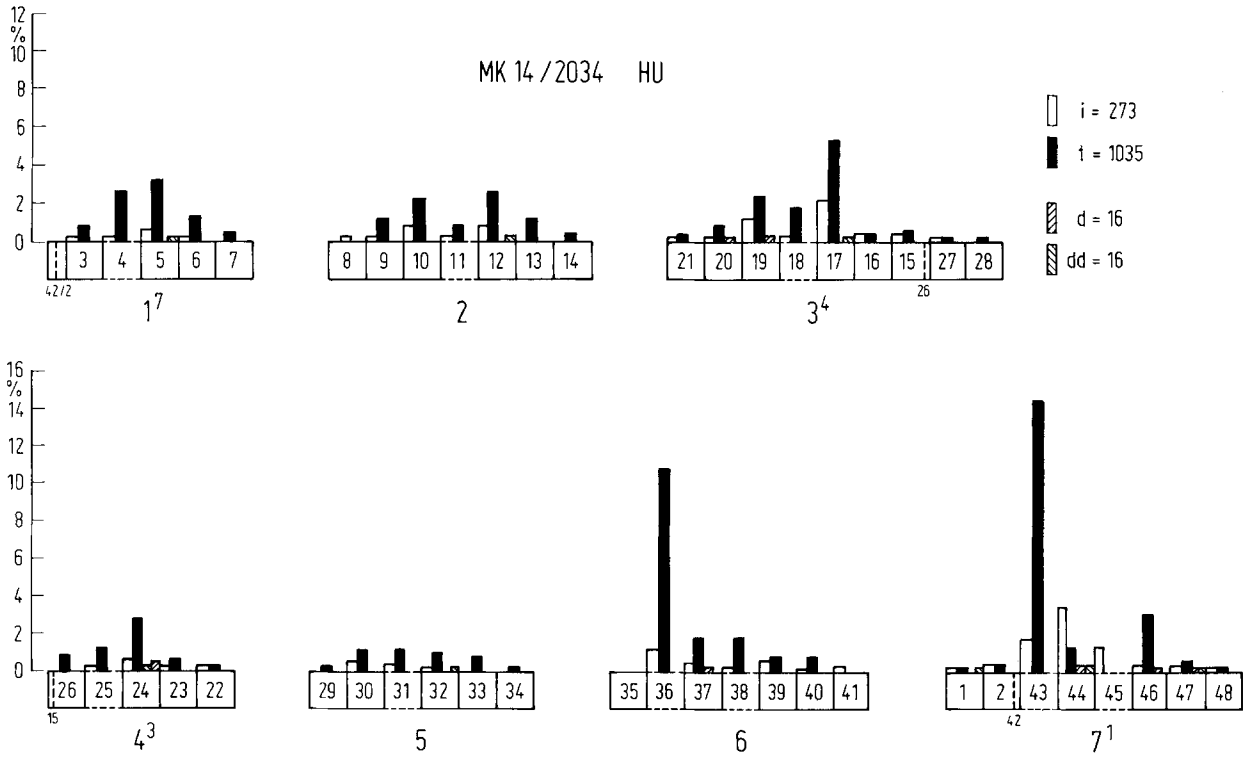

Fig. 1. The percent involvement in chromatid aberrations (isochromatid breaks = i; duplication-deletions = dd; intercalary-deletions = d; lesions involved in chromatid translocations = t) of the karyotype MK 14/2034 subdivided into 48 segments after treatment with HU. Segments 36 (NOR of chromosome 6) and 43 (NOR of chromosome 7) are aberration "hot spots". The chromatid exchanges localized in these segments comprise 10.8% and 14.6% respectively of all the aberrations scored. (Parallel control to experiments of Fig. 2)

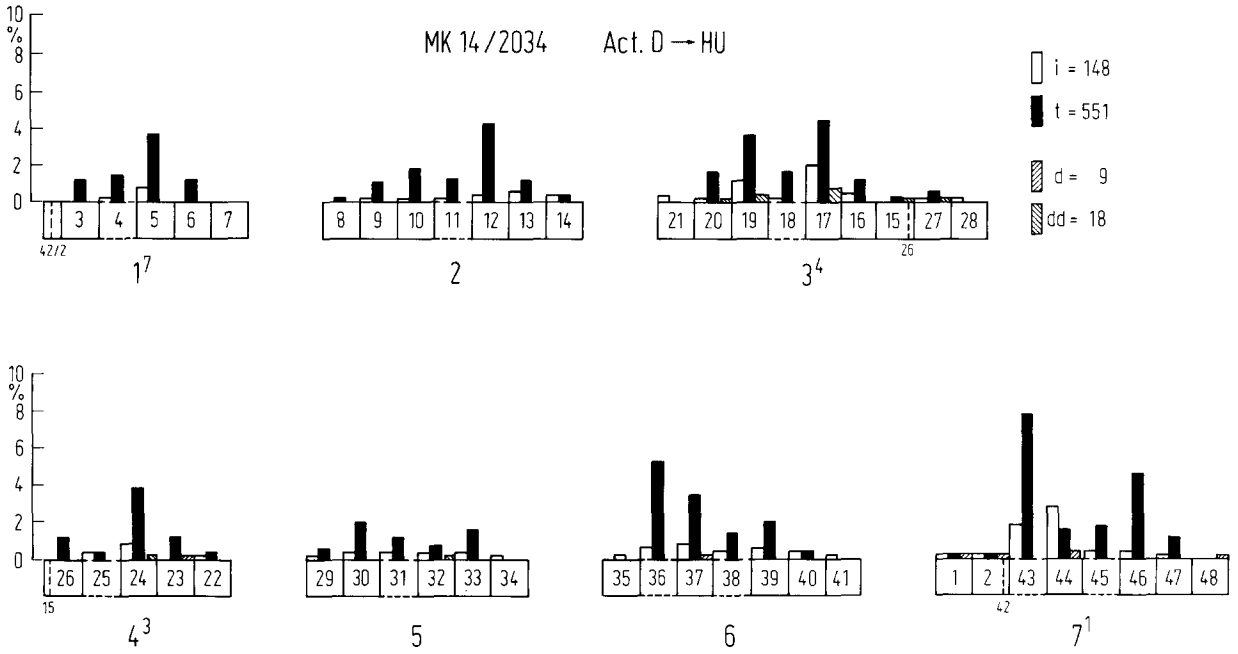

Fig. 2. Effect of Act D applied as a prolonged pretreatment (19 h) before the treatment with HU. The frequencies of chromatid exchanges in segments 36 and 43 are approximately a half lower (5.2% and 7.6%) as compared to the control-treatment with HU alone

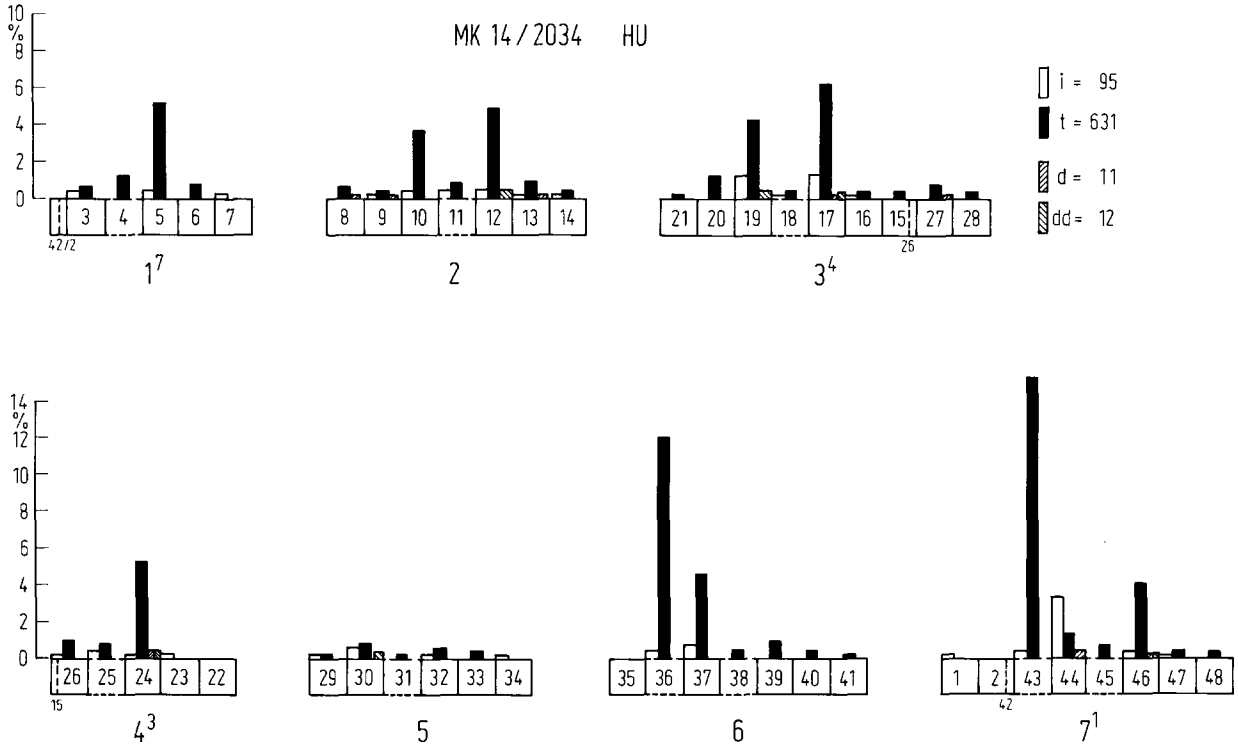

Fig. 3. The percent involvement in chromatid aberrations after treatment with HU. Chromatid exchanges localized in segments 36 and 43 comprise 12.0% and 15.6% respectively. (Parallel control to experiments of Fig. 4)

Statistically significant clustering of breaks and/or chromatid translocations also occurred in segments 5, 12, 17 and 24. The frequencies of aberrations in these segments however were just at the upper confidence limits of the length proportional aberration distribution (1% level). This means that these segments represent no real HU-induced aberration hot spots as compared to segments 36 and 43.

Act D applied separately at the low concentration of $4 \cdot 10^{-8}$ M (0.05 µg/ml) did not show any mitotic blockade or chromosome changes in the meristem cells of barley root tips. However, when applied at this concentration before the mutagen or simultaneously with the mutagen, Act D exerted an apparent effect on HU-induced aberration frequencies in segments 36 and 43. Figure 2 shows the effect of Act D applied as a prolonged pretreatment (19 h) before the mutagen treatment (HU was added at zero time after Act D pretreatment). As seen from Fig. 2 the frequencies of chromatid exchanges in segments 36 and 43 proved to be approximately a half lower (5.2% and 7.6%) as compared to the respective control – treatment with HU alone (10.8% and 14.6%, Fig. 1). The frequencies of aberrations in both segments after application of Act D did surpass the upper limits of confidence intervals (22) but not more than one or twofold. This indicated that these segments still remained aberration hot spots.

The tests of varying the time of Act D pretreatments indicated that a similar decrease in mutation frequencies also occurred in segments 36 and 43 when Act D was applied 3 to 6 h before the immediate treatment with HU.

The same effect was observed after simultaneous application of Act D and HU for 6 h (Fig. 4). The frequencies of chromatid exchanges in segments 36 and 43 were again approximately twofold lower in this case (5.0% and 8.8%, Fig. 4) as compared to the control (12.0% and 15.6% respectively, Fig. 3). These frequencies however did surpass the upper limits of confidence intervals (22) but also not more than one or twofold, characterizing them still as aberration hot spots.

We next tested whether a prolonged pretreatment of 15 h with Act D followed by a subsequent Act D-free recovery period of 5 h before mutagen treatment would exert any influence on aberration clustering in segments 36 and 43 (Fig. 6). As the figure shows, a marked clustering of chromatid translocations in these segments occurred after such pretreatment condition. The aberration frequencies in segments 36 and 43 proved to be again practically as high as in the respective control – treatment with HU alone (Fig. 5). It appears therefore that a recovery period of 5 h inserted between the prolonged Act D pretreatment and the mutagen treatment, completely eliminated the effect of the drug, i.e. the cells could recover their original mutagen sensitivity upon transfer into a drug-free medium.

Under all these treatment conditions changes in aberration involvement were not limited to segments 36 and 43 only. After prolonged Act D pretreatment (19 h)

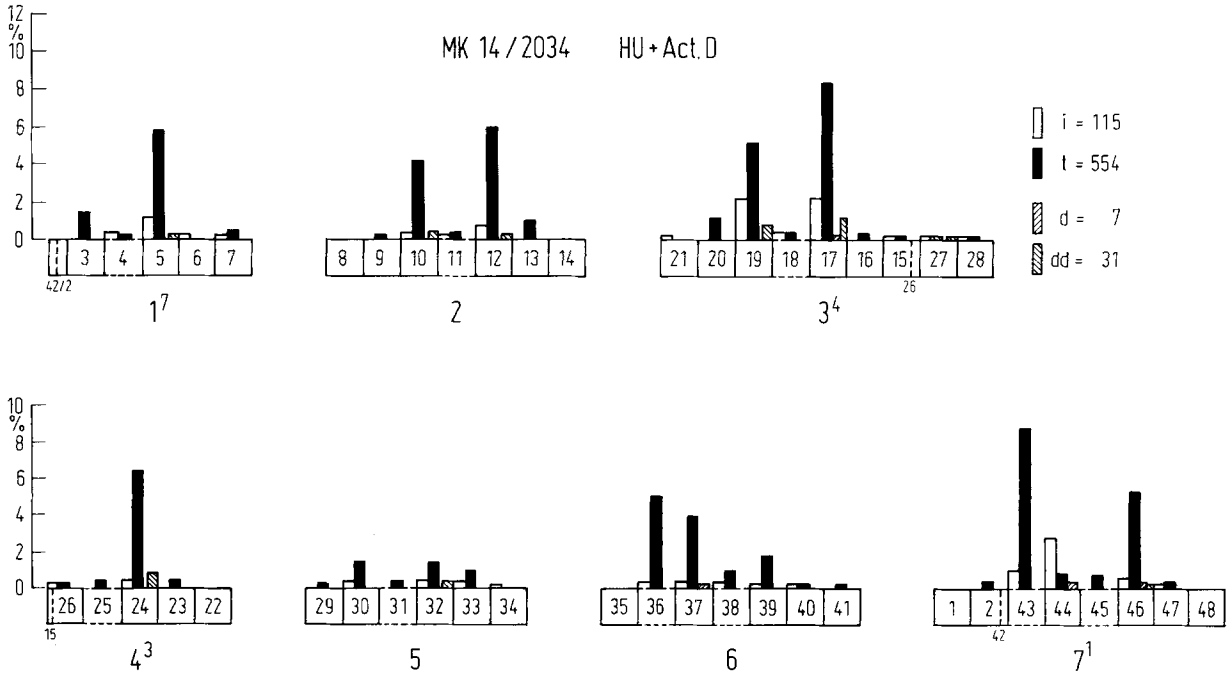

Fig. 4. Effect of simultaneous application for 6 h of Act D and HU. The frequencies of chromatid exchanges in segments 36 and 43 are approximately twofold lower (5.0% and 8.8%) as compared with the control-treatment with HU alone

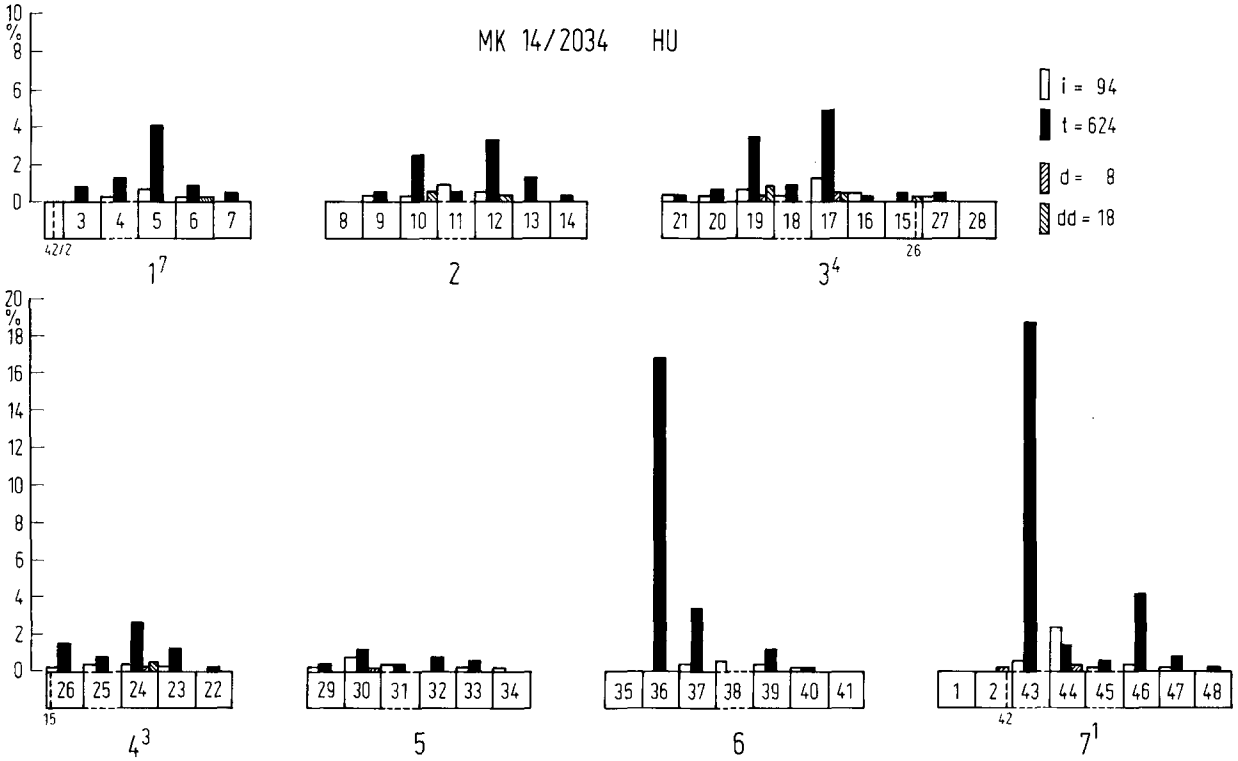

Fig. 5. Chromatid aberration distribution after treatment with HU. Translocations localized in segments 36 and 43 comprise 16.3% and 18.4% respectively. (Parallel control to experiments of Fig. 6)

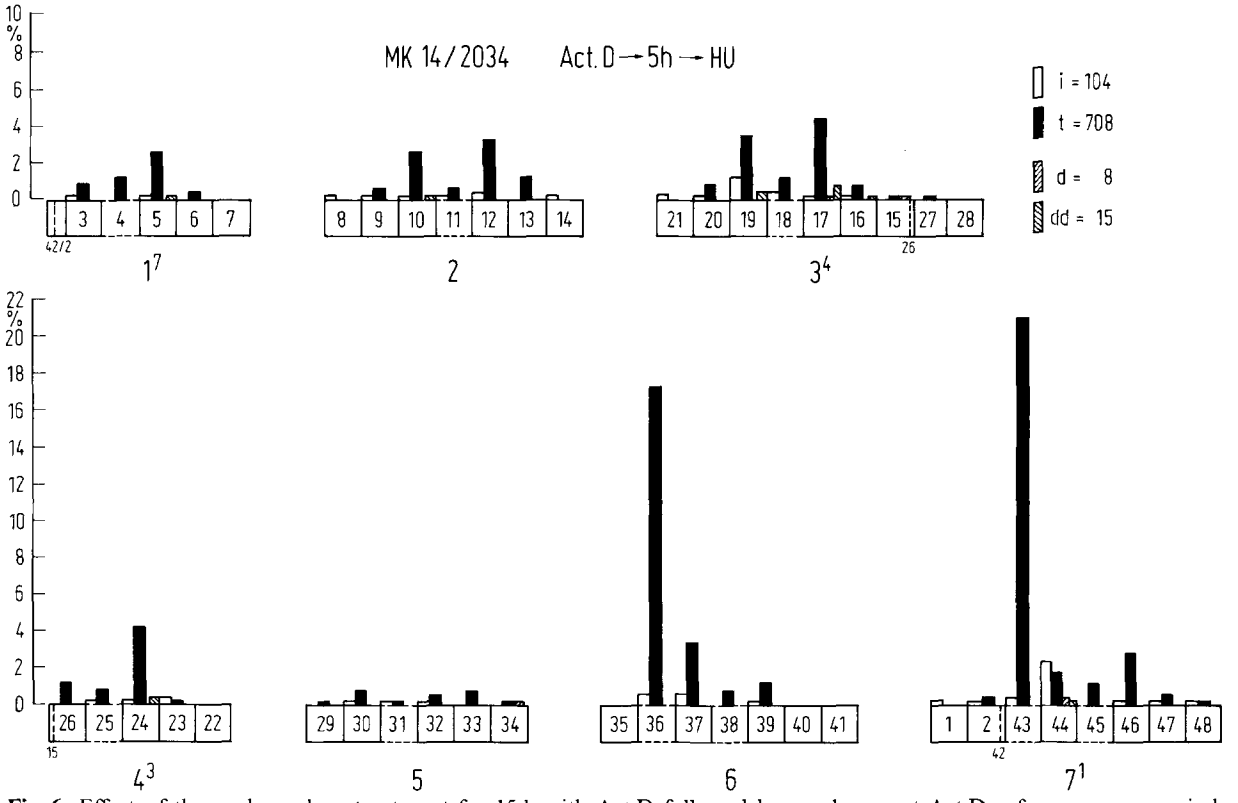

Fig. 6. Effect of the prolonged pretreatment for 15 h with Act D followed by a subsequent Act D – free recovery period of 5 h before treatment with HU. Translocations localized in segments 36 and 43 comprise 16.4% and 20.8% respectively i.e. practically as high as in the control-treatment with HU alone

or combined treatment with Act D and HU (6 h) and parallel with the decrease in mutation frequencies in segments 36 and 43, some slight but statistically valid increase of aberration frequency of segments 33, 37 or 39 was also observed ($\alpha < 5\%$). It appears that the reduced expression of the aberration hot spots become compensated for by a concomitant increase of aberration frequency in other segments for reasons that are not yet understood. This fact is likely to have much in common with the similar observations made by Kaina et al. (1979) and Schubert et al. (1981) in *Vicia faba.*

Discussion

In previous studies (Nicoloff et al. 1977, 1979; Anastassova-Kristeva et al. 1977, 1979) it has been shown that, due to chromosome structural changes of barley karyotype, NORs may become translocated from their original sites. This results in certain cases in modifications of the synthetic activity of rRNA cistrons in NORs, respectively in the normal formation and morphology of the nucleoli. Thus, combination by means of translocation of all four NORs into a single barley chromosome pair, i.e. two NORs per chromosome, either in opposite arms or tandemly in one chromosome arm of satellite chromosomes, was found to result in complete or partial repression of the rRNA cistrons. This phenomenon has already been inferred from impaired nucleolus formation by two of the NORs. It has also been found that the NOR of satellite chromosome 6 is the one which forms the larger nucleoli, i.e. shows a standard rate of transcription, while the NOR of satellite chromosome 7 remains suppressed in all cases of NORs combination by interchange in a single chromosome pair (Anastassova-Kristeva et al. 1979, 1980). Though the mechanism underlying this phenomenon is still unknown the cases represent a position effect with respect to the transcription of rDNA.

Further, a close correlation between the different rate of synthetic activity in nucleolus formation and the comparable range of variation in mutation involvement of NORs was observed (Nicoloff 1981). Thus, the decreased synthetic activity in the nucleolus formation of NOR of chromosome 7 was found to be invariably connected with a significant decrease in the amount of aberrations induced in this NOR. Therefore, the data obtained from these studies provided some lines of evidence which indicated dependence of chromosome mutation induction in NORs upon transcriptional activity of rDNA.

Based on these findings it would therefore be desirable to ascertain whether an experimentally induced selective inhibition of rDNA transcription could also display changes in mutation involvement of NORs similar to the changes observed in the translocation karyotypes mentioned above.

The present studies showed that a low concentration of 0.05 µg/ml Act D applied before or simultaneously with HU decreases the frequencies of chromatid aberrations in NORs. This suggests a possible connection between the mutation induction and the transcriptional activity of NORs, since Act D at a low concentration is known to be a highly selective inhibitor of rRNA transcription (Perry 1962, 1963; Perry and Kelley 1970).

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Book Reviews

Vogel, F.; Propping, P.: Ist unser Schicksal mitgeboren? Moderne Vererbungsforschung und menschliche Psyche. Berlin: Severin und Siedler 1981. 368 pp., 60 figs. Hard bound DM 42,–.

The authors are rather competent to write a book on human behaviour genetics and have very well succeeded in doing so. Why do they give the book such an uninteresting title: Is our fate congenital? What fate? But the point to be made is: Are my doings hereditary? This marvelously interesting book concedes nothing either in the title nor in the text to potential lay-readers. You have to be a rather educated and stubborn reader to finish it. About 340 pages of clear expositions are quite a bit for a superficial lay-geneticist, thinking about his schizophrenic sister or his mother, who died from carcinoma of the breast at 48 years. The first example is discussed at length, but the second fate, if congenital, is missing.

In 17 chapters genetics and man's psyche are discussed and chapters 3 and 17 are the most exciting, giving some personal views on social darwinism (chapter 3), and a preview on a genetic blessing or curse (17). It is regrettable that sociobiology (Wilson a.o.) is not mentioned, nor newer biological viewpoints like those on cybernetical systems. The sociobiological point of view would have raised discussions on the psychological, sociological, ethical, political and even philosophical implications of genetics. The cybernetical aspect would have made it clear, that at least in biological systems we have to deal with action and reaction in a reciprocal system and not with a simple cause and effect relation only.

There are many non-Germans who emotionally reject discussions by Germans on normal and ethical implications of human genetics in the past (social darwinism, racism) and the future. A cautious discussion of the burden of the knowledge of the future genetic fate of an individual and the reactions of the society to this knowledge ("good and bad fairy") belongs to the responsibility of the scientists. It is realistic and acceptable that individual scientists do so and it is not right to reject these views on past mistakes and crimes of fellow countrymen. Society has to come to grips with the predictability of individual genetic risks and interdisciplinary and international discussions are necessary to find solutions to the stigmatization and the insurance problems.

The present book gives a very good overview of human behaviour genetics and geneticists as well as non-geneticists should use it as a basis for interdisciplinary discussions.

S. J. Geerts, Nijmegen