Effect of Different Concentrations of 5-Fluordeoxyuridine on Mitosis and Chromosomes of Barley Root Meristems

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Abstract. Excised barley embryos were cultivated in a liquid complete medium for 48 h and then 5-fluordeoxyuridine was added. The concentrations of 5-fluordeoxyuridine differed in the range of 6 orders of magnitude (10⁻⁸ to 10⁻³ M). All concentrations, except 10⁻⁸ M led to a total inhibition of mitosis during 24 h. 5-fluordeoxyuridine induced chromosomal aberrations of the non-exchange type in all concentrations used. The frequencies of induced breaks increased during the interval of 12 h in which they were followed and, in samples fixed at later intervals after the beginning of the treatment, there was a tendency to clustering of the induced fragments in some mitotic figures. The most striking feature of the effects observed is the relatively small dependence on the concentration used.

5-fluordeoxyuridine is, in low concentrations, a very specific inhibitor of DNA synthesis. It arrests cycling cells in the S phase. The mechanism of action of FUdR was described at first in E. coli by Cohen et al. (1958). FUdR is phosphorylated by thymidine kinase and FUdR monophosphate inactivates thymidilate synthetase irreversibly and so blocks the conversion of deoxyuridin monophosphate to thymidin monophosphate. The inhibition of DNA replication in eucaryotes takes place in very low concentrations of FUdR: 10⁻⁸ M—10⁻⁶ M (Kihlman 1966). If applied in higher concentrations, FUdR can be metabolized to 5-fluorouracil and this is incorporated into RNA, which leads to the inhibition of translation (Heidelberger 1963).

FUdR causes inhibition of mitosis even in very low concentrations (Kihlman 1962, Hsu et al. 1964). Low concentrations of this drug are able to induce chromosomal aberrations (Bell and Wolff 1964, Taylor et al. 1962). These are of the non-exchange type, i.e. they consist of free breaks and gaps only.

The dependence of the mitotic inhibition and induction of breaks by FUdR on the concentration of the drug was never studied systematically. The purpose of this paper was to study the effects of different concentrations of FUdR on the mitotic inhibition and induction of chromosomal aberrations.

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348
MATERIAL AND METHODS

Spring barley *Hordeum vulgare* cv. Ametyst, fraction with seed diameter 2.5–2.8 mm was used throughout the experiments. Embryos were extirpated under aseptic conditions and cultivated in the liquid complete medium of Gamborg *et al.* (1968) on a gyratory shaker by the method already described (Gichner *et al.* 1976). Embryos were kept in the dark during cultivation, in temperature 26 ± 1 °C. After 48 h cultivation, FUdR (Serva) was added to make final concentration range 10^{-3} M to 10^{-8} M. The material was fixed in ethanol acetic fixative (3 : 1) at regular intervals from the beginning of the treatment by FUdR (1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h, respectively). Squash slides, stained by Feulgen's method were made. The MI was estimated on the basis of evaluation of 5 slides, 1000 nuclei were scored on each. To estimate the frequencies of aberrations, 500 metaphases and 500 anaphases were scored with a few exceptions; when the mitotic index was very low, the number of mitotic figures scored was somewhat lower, but never under 200. To make scoring aberrations in metaphases possible, colchicine was added to the cultivation medium to make the resulting concentration 0.1%, the last two hours before fixation.

RESULTS

**Suppression of Mitosis by FUdR Treatment**

During the first two hours after addition of FUdR to the nutrient solution there were no statistically significant differences of MI values among variants of the experiment (Fig. 1), but starting from 4 h after the addition of FUdR MI showed a progressive decrease till the end of the experiments. The time

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*Abbreviations used:* FUdR = 5-fluorodeoxyuridine; MI = mitotic index.
course of the decrease was the more pronounced the higher was the concentration of FUdR. After one mitotic cycle time, which is under our experimental conditions about 12 h (Schwammenhöferová and Ondřej 1978), FUdR caused a statistically significant decrease in all concentrations under study. After 24 h treatment by FUdR, MI was always very low (under 2%),

with the exception of the treatment by 10⁻⁸ M FUdR where the decrease of MI value was just on the verge of statistical significance against control. In 10⁻³ M FUdR, MI was lower than in other experimental variants from the beginning of the experiment and after 10 h and later it was beyond scoring.

**Induction of Chromosomal Aberrations**

The most striking feature of the induction of chromosomal aberrations by FUdR was that there were no great differences in the frequencies of aber-
rations induced after application of FUdR in concentrations which differed in six orders of magnitude.

FUdR induced only chromosomal aberrations of the non-exchange type, i.e. fragments in anaphase and isolocus breaks, chromatid breaks and terminal deletions in metaphase figures. The same picture was found earlier by other authors (Bell and Wolff 1964, Kihlman 1962). The frequency of induced chromosomal breaks increased gradually with increasing duration of exposition to FUdR (Fig. 2). In later fixation periods, there was a tendency to the nonrandom distribution of the frequency of breaks between individual mitotic figures. Most commonly, there were either no breaks or several breaks. Mitotic figures with one or two breaks were less frequent than would be expected on the basis of random distribution.

In the early fixation periods (up to 6 h) the frequencies of induced breaks increase with increasing concentration. In the later periods (8–12 h from the beginning of FUdR treatment) the frequency of breaks after \(10^{-4}\) M FUdR tends to be lower than that after \(10^{-5}\) M FUdR. Perhaps the nuclei which were most severely damaged do not reach mitosis at all and this leads to a decrease of the frequency of breaks in those cells which did appear in mitosis.

Chromosomal breaks were scored in individual samples up to 10 h in metaphases and up to 12 h in anaphases. In the samples fixed 24 h after the onset of the treatment, MI was very low and scoring of chromosomal aberrations therefore impossible. At 1 h interval, only anaphase aberrations and no metaphase aberrations were scored as it is not possible to make 2 h colchicine pretreatment in this variant of the experiment.

DISCUSSION

Effect of FUdR on Mitotic Cycle

FUdR blocks cells in the S phase. When root meristems are permanently immersed in FUdR solution, only those cells which were in G2, or perhaps on the verge of G2 and S appear in mitosis and a proportion of the later shows chromosomal breaks. The course of the mitotic cycle in the root meristems of the barley embryos cultivated by our method has already been followed (Schwammerlhöferová and Ondřej 1978). The average duration of G2 was estimated to 6–8 h and the minimum G2 duration (or shortest G2, in a low proportion of cells) was 2–3 h. Supposing that the primary cause of the gradual decrease of the mitotic cycle is the blockage of cells in the S phase, the kinetics of the mitotic cycle explains why the first decrease of MI was observed 4 h from the beginning of the treatment. It corresponds to the sampling of cells which were at the end S at the beginning of the treatment. The gradual decrease of MI up to 12 h after the beginning of the treatment shows that some cells are either naturally or as a consequence of FUdR treatment arrested in G2 for as long as 12 h. Of course, there is also a second possibility, that some cells leave S, with a limited velocity, even in the presence of the drug.

It can be supposed further that the velocity of leaving S is reversely related to FUdR concentration. The third supposition we can make is that only a proportion of those cells which were already in the S phase at the beginning of the treatment can leave S in the presence of FUdR, i.e. the
S → G₂ transition is blocked. It could explain why after 24 h treatment MI in all FUdR concentrations except 10⁻⁸ M was near to zero. In the 10⁻⁸ M FUdR, perhaps replication was not yet severely inhibited and the G₁ → S transition was not blocked, only the duration of S was probably increased.

This model of the action of FUdR can also explain why MI decreased by a different time course, or by different speeds which increased with increasing concentration.

The Induction of Chromosomal Aberrations

Chromosomal fragments are induced by FUdR most probably in cells which were near the end of the S phase at the beginning of the treatment. FUdR induced inhibition of replication caused the exit of the cells from S with one or a few sites nonreplicated. If the replication fork was damaged and if the uninnomodel of the chomosoma structure holds true, it explains why isolocus breaks were the most common type of aberrations found in metaphases. This damage of cells which were at the end of S at the beginning of the treatment explains why there were numerous cells with multiple fragments. The probability that the beginning of the treatment of FUdR meets cells with replication of just the last replicon unfinished is low; replication of several replicon unfinished is more likely.

Repair of the DNA damage and of chromosomal aberrations induced by FUdR does not occur, because DNA synthesis is inhibited.

As seen in Fig. 2, the frequency of chromosomal aberrations in metaphases is relatively higher than that in anaphases. When the spectrum of chromosomal aberrations is broad, there are some types of aberrations detectable in metaphases which escape detection in anaphases and this results in higher efficiency of detection of aberrations in metaphases. In our experiments with the induction of chromosomal aberrations by X rays (ONDŘEJ and LIJK EN SE 1976) the frequency of chromosomal aberrations in metaphases was only very slightly higher than in anaphases. In the results presented here, however, only chromosomal aberrations of the non-exchange type were induced and the possibilities of their detection in metaphases and anaphases are equal. On the other hand, in metaphases, a very high degree of chromosome fragmentation-pulverization of chromosomes is met accidentally, after FUdR treatment. These severely damaged cells are unlikely to reach anaphase and perhaps they are the source of higher values of aberrations in metaphases. Perhaps in metaphases also some gaps were scored together with chromatid breaks and perhaps in anaphases, some fragments were masked by the clumped anaphase chromosome sets. All three mechanisms could also participate together.

REFERENCES


5-FLUORODEOXYURIDINE AND MITOSIS


BOOK REVIEW


Thirteen chapters of this book, written by prominent authors from eight countries, are divided into three parts. Three chapters of the first part deal with the structure of guard cells, their walls, chloroplasts, vacuoles, other organelles and with substomatal ion-adsorbent bodies (R. A. Stevens and E. S. Martin); stomatal ontogeny and classification in some dicotyledonous families with special emphasis on taxonomic and phylogenetic significance (B. Kannabiran and K. H. Krishnamurthy) and on stomatal features of Acanthaceae, which may help in a better understanding of the taxonomy of this large pantropical family of flowering plants (K. J. Ahmad).

Part two is devoted to the function of stomata and contains five chapters. The first deals with the structure of guard cell chloroplasts, their photosynthetic capacity and with the hypothetic destiny of carbon dioxide in guard cell metabolism and functions (S. Lurie). The second chapter clears up the role of abscisic acid in stomatal regulation of gas exchange in relation to environmental and internal factors and plant development (B. R. Loveys and F. E. Kriedemann); the third chapter reviews the role of distinct groups of growth regulators in stomatal function (R. F. Horton). The next chapter discusses the guard cell metabolism predominantly on the results obtained from model plant material Commelina (F. L. Milthorpe, N. Thorpe and C. M. Willmer). The last chapter of this section deals with causal interpretations of stomatal response to chemicals, which may affect stomatal movements by altering guard cells membrane permeability, K+ balance, starch metabolism, glycolate metabolism and by disturbing CO2 concentration in plant tissue (M. A. Pemadasa).

Part three devoted to ecology of stomata is introduced by the chapter on the versatile role of stomata in coordinating plant needs (H. Moldau). The chapter on the effect of changes of ambient air humidity on stomatal responses and their interrelations with other environmental factors (R. Lüösch) is followed by the chapter on peculiarities of stomatal regulation in some important arid zone plants (D. N. Sen, D. D. Chawan and R. P. Bansal). The last but one chapter is devoted to differences in response of adaxial stomata to environmental variables (N. C. Turner). The concluding chapter reviews the present knowledge of circadian rhythms in stomatal movement (E. S. Martin and R. A. Stevens).

The volume provides a useful basic account of the topic and it is strongly recommended to all who are concerned with stomatal physiology, ecology and structure and with processes depending on or connected with stomata actions.

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