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Participation of Exogenous DNA in the Repair Processes of Meristematic *Vicia faba* Cells Injured by Monofunctional Alkylating Agens Ethylmethane Sulphonate

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Abstract. The effect of exogenous DNA of syngeneic origin on the course of reparation of meristematic cells of *Vicia faba* primary roots followed after treatment with monofunctional alkylating agent, ethyl methanesulphonate (EMS), was tested. Time course of alternations in mitotic activity of investigated cellular population and the dynamics of formation of postmetaphase chromosomal aberrations was evaluated. A reparation of damaged cells was significantly supported by syngeneic DNA; its application induced an increased incidence of cellular division already in the early intervals of the repair which was accompanied by concomitant decrease of microscopically detectable rupture in the chromosomes. The study performed on the localization of induced damages occurring in metaphase chromosomes pointed out an increased sensitivity of small chromosomes of *Vicia faba* to EMS. Similarly, a reparative action of syngeneic DNA was exhibited by significant decrease of aberrations frequency, predominantly in the same chromosomal group. Per cent representation of individual types, not affected by the action of syngeneic DNA, was established by detailed classification of induced aberrations. In both cases, isochromatide breaks were found of greatest predominance.

Our preceding reports (ŠLOTOVÁ and KARPFEL 1968, 1969) have brought an evidence on favorable effect of exogenous DNA of syngeneic origin on the repair processes occurring in meristematic cells of *Vicia faba* primary roots after treatments with various mutagens of physical and chemical nature. However, in the case of chemomutagens action a repair effect of syngeneic DNA was variously exhibited.

It has been shown that its participation in the repair processes is probably conditioned by a mechanism of the action of acting agens. While in the case of bi- alkylating substance Ypenyl (ŠLOTOVÁ and KARPFEL 1969), a reparation of injured cellular population was significantly accelerated by syngeneic DNA; it was effectless in the case of the use of maleic hydrazide radiomimetics (ŠLOTOVÁ *et al.* 1971). On the basis of mentioned findings it may be supposed that syngeneic DNA is employed only in the cases when

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an acting chemomutagen immediately interferes with the synthesis of recipient's DNA. Presented report completes our earlier results with evaluation of the action of exogenous DNA on the course of reparation followed at the same experimental material, the meristematic cells of *Vicia faba* primary roots, after the action of ethyl methanesulphonate, monofunctional alkylating derivative. Attention was focused predominantly to time-course of alternations in mitotic activity of investigated cellular population and to a dynamics of formation of postmetaphase chromosomal aberrations. Furthermore, we were interested in detailed interchromosomal distribution of induced damages; their analyses in the metaphase chromosomes were also made.

Material and Methods

As an experimental material, the meristematic cells of *Vicia faba* primary roots (cv. Považský used at the 6th day after germination according to GRAY and SCHOLES 1951) were employed.

Ethyl methanesulphonate (EMS) (Koch-Light Laboratories Ltd., Colnbrook, Bucks., England) was used in Sörensen phosphate buffer (0·017 M at pH 7·0, concentration at 100 mmol l^{-1}).

Radiomimetics action lasted for 1, 2, 4 h: a recovery required 16, 24 and 48 h periods and was carried out both in tap water and solution of syngeneic DNA.

Syngeneic DNA isolated from *Vicia faba* cv. Považský roots was obtained according to the method of KNIGHT (1952). The DNA preparations contamined with not more than 1% of RNA and 0·7% of proteins was used at concentration of 10 μg ml^{-1} 0·01 M NaCl and was of native character.

After EMS action and after an appropriate recovery *Vicia faba* root tips were fixed and squash slides stained by Feulgen method were prepared. Mitotic activity of studied cellular population, expressed by the values of mitotic index (MI designates per cent part of dividing cells) as well as frequency of chromosomal aberrations in the meta- and postmetaphases (ana- and telophases), were evaluated. Concomitantly, a distribution of induced abnormalities between groups of large and small *Vicia faba* chromosomes was also established. In metaphase chromosomes (*i.e.* after 2 h treatment with 0·05% colchicine solution) an analysis of a type of injury induced by the action of EMS in *Vicia faba* chromosomes was performed as well.

An evaluation was made in the similar way used in our earlier contributions (ŠLOTOVÁ and KARPFEL 1968 and others).

All experiments were carried out at 20 °C, and were repeated and statistically evaluated.

Results

1. The Changes of Mitotic Activity of Meristematic Cells After the Action of Alkylating Agens

This group of experiments brings evaluation of the changes in mitotic activity of meristematic cells population induced by differently lasting treatments of EMS at concentration 100 mmol l^{-1} (1, 2, 4 h). Observed changes expressed by mitotic index values in dependence partly on duration of the recovery (16, 24, 48 h) and partly on its type (water, syngeneic DNA resp.) are given at Fig. 1. It is obvious that due to EMS action a decrease of number of dividing cells in the studied population occurs. Such an inhibition is proportionally dependent on a duration of treatments with radiomimetics usage. During the course of recovery, a gradual regeneration of cellular division occurs, as shown by relative increase of mitotic index values. It is also demonstrated at Fig. 1 that a transport of (by EMS) damaged roots into syngeneic DNA solution of 10 μg ml^{-1} concentration leads to a more rapid repair of cellular division in all time intervals tested.

TABLE 1

EFFECT OF SYNGENEIC DNA on the Interchromosomal Distribution of Aberrations after Variously Lasting Action of EMS at Concentration 100 mmol l^{-1}.

Duration of EMS action [h]	Type of recovery 24 h	Number of damages in group of large chromosomes M	Number of damages in group of small chromosomes m	Ratio of the damages $M : m$
1	water	22	78	1 : 3.54
	DNA	45	57	1 : 1.26
2	water	28	95	1 : 3.39
	DNA	51	68	1 : 1.33
4	water	19	69	1 : 3.63
	DNA	37	52	1 : 1.40

2. The Frequency of Postmetaphase Chromosomal Aberrations

An incidence of postmetaphase aberrations (*i.e.* ana- and telophase ones) was determined in different time intervals (16, 24, 48 h) after 1, 2, and 4 h treatments with 100 mmol l^{-1} EMS solution. As illustrated at Fig. 2, the longer lasting EMS action on *Vicia faba* roots, the higher frequency of the cells with damaged chromosomes is observed. At Fig. 2 time dependence of the action of tested chemomutagens is also given; with a progress in the recovery, per cent of the damage is lowered. In the case of damaged roots reparation performed in syngeneic DNA solution (10 μg ml^{-1}), a lower incidence of chromosomal aberrations was received in all experimental groups. Thus, it is clear that a significant reparative effect of the DNA of syngeneic origin is exhibited.

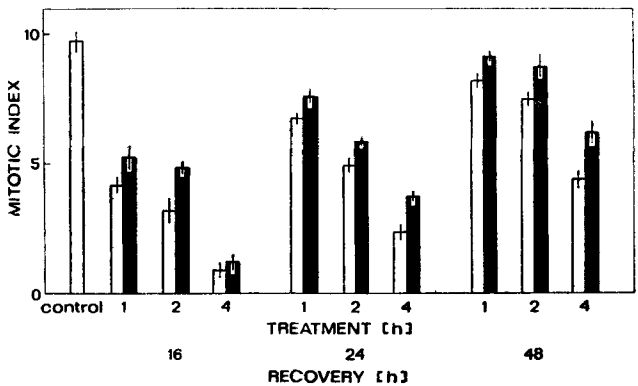

Fig. 1. Effect of exogenous DNA of syngeneic origin on the changes of mitotic activity of *Vicia faba* meristematic cells induced by variously lasting EMS action. Abscissa: duration of EMS action given [h]; time interval of recovery [h]. Ordinate: mitotic index (*MI*) values. Controls; open columns — recovery in water, black columns — recovery in the syngeneic DNA.

3. Interchromosomal Distribution of Aberrations

In the meristematic cells of *Vicia faba* a distribution of induced abnormalities among groups of large and small chromosomes of *Vicia faba* (performed similarly after 1, 2, and 4 h. of EMS action at 100 mmol l^{-1} con-

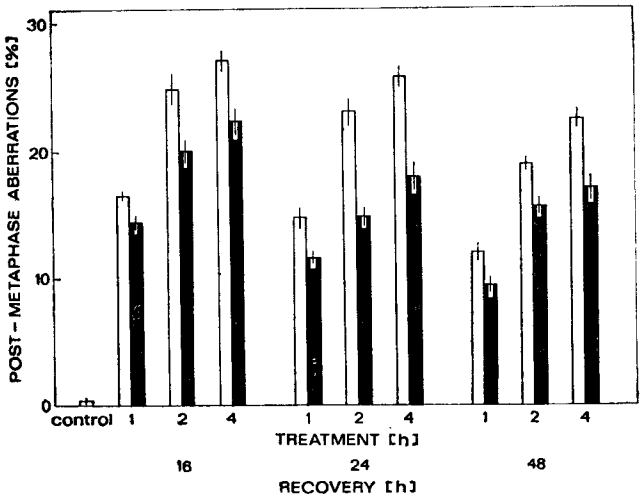

Fig. 2. Dependence of frequency of chromosomal aberrations induced by 1, 2 and 4 h treatment with EMS upon duration and type of the recovery. Abscissa: duration of EMS treatment [h]; time interval of recovery [h]. Ordinate: per cent of postmetaphase chromosome aberrations (*Ab*). Controls; open columns — recovery in water, black columns — recovery in the syngeneic DNA.

centration and after 24 h recovery in water, or in syngeneic DNA at 10 $\mu g\, ml^{-1}$ resp.) was examined. For these purposes, mitotic division of the population was discontinued in the metaphases using a treatment with 0.05% colchicine solution applicated in the latter 2 h of an appropriate time interval. Obtained results are summarized in Table 2 indicating an increased sensitivity of small chromosomes of *Vicia faba* against EMS. Reparative effect of syngeneic DNA was also manifested, predominantly in the same chromosomal group. This is understood in the small chromosomes where an application of mentioned biomacromolecule leads to a substantial decrease of incidence of aberrations.

4. Classification of a Type of Induced Aberrations

Latest experimental group was devoted to determining an incidence of chromatide aberrations or of chromosome ones respectively, in the meristematic cells induced by EMS. Induced aberrations were randomly of chromatide type, similarly as in the case of the most of chemomutagens. Per cent expression of representation of individual aberration types is illustrated at Fig. 3. Isochromatide breaks (*B''*) greatly predominate; free chromatide breaks (*B'*) are found less often. As shown later on at Fig. 3, syngeneic DNA exogenously applicated does not affect a relative appearance of individual types of chromosomal aberrations in the meristematic cells of *Vicia faba* damaged by EMS.

Discussion

Our preceding reports have brought an evidence on the ability of exogenous DNA of syngeneic origin to affect favorably the repair processes of the cells injured by mutagen of physical nature — by ionizing radiation (ŠLOTOVÁ and KARPFEL 1968) as well as by a chemical mutagen — bifunctional alkylating substance Ypenyl (ŠLOTOVÁ and KARPFEL 1969). Presented con-

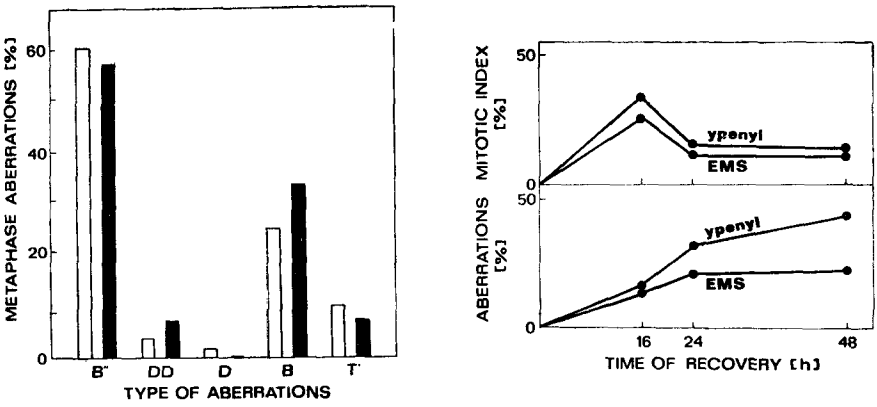

Fig. 3. Effect of syngeneic DNA on the type of aberrations induced by EMS. Abscissa: type of aberrations — *B''* — isochromatide breaks, *DD* — duplication-deletions, *D* — intercalary deletions, *B'* — chromatide breaks, *T'* — chromatide translocations. Ordinate: per cent representation of individual types of aberrations. Open columns — recovery in water, black columns — recovery in the syngeneic DNA.

Fig. 4. Rate of repair effect of syngeneic DNA [%] after the action of monoalkylation (EMS) and bialkylation (Ypenyl) substance. Abscissa: time interval of recovery [h]. Ordinate: per cent increase of mitotic activity (*MI*), or a decrease of frequency of induced aberrations (*Ab*) resp., influenced by syngeneic DNA (10 μg ml^{-1}).

tribution is aimed to continuation and evaluates a participation of syngeneic DNA in the repair processes of meristematic cells of *Vicia faba* injured by monofunctional alkylating substance ethyl methanesulphonate (EMS).

It has been already demonstrated by LOVELESS (1951) that monofunctional derivatives of alkylating substances may induce aberrations in the chromosomes of *Vicia faba*, not distinguishable from injuries induced by bi-functional analogons. However, it has been shown by further cytogenetic studies made with monofunctional alkylating substances that their activities are limited by certain concentration range. For example, according to RIEGER and MICHAELIS findings (1960), long lasting EMS exposures are effective only at higher concentrations convergating to a treshold value over which a complete break of cellular division occurs. On the basis of these results, concentration of EMS adjusted at 100 mmol l^{-1} was considered as the most suitable. It was demonstrated that a remarkable depression of cellular division accompanied by a formation of chromosomal aberrations, mostly of chromatide type, occurs in the meristematic cells of *Vicia faba* roots in dependence on the duration of the treatment with mentioned chemomutagen solution and the duration of the recovery. The damages which arose are

localized mainly in small *Vicia faba* chromosomes, probably in their heterochromatine segments. During the course of recovery, partially spontaneous reparation of induced damage occurs (*cf.* Figs. 1 and 2). In the case of (by EMS) damaged roots cultivated in a solution of exogenous DNA of syngeneic origin, a significant strenghthening of reparation occurs: already in the early interval of the recovery, an increased incidence of cellular division accompanied with concomitant decrease of microscopically detectable injuries of the chromosomes may be observed. It is therefore apparent that also in this case, syngeneic macromolecule of DNA actively participates in the repair processes. Fig. 4 represents relative repair effect of syngeneic DNA in the meristematic cells of *Vicia faba* affected by monofunctional alkylating chemomutagen (EMS) comparing it with a bi-functional agens Ypenyl. It is pointed out that, at reparation of mitotic activity, maximal effect is achieved in 16 h interval of the recovery, regardless to a number of functional groups of the chemomutagen used. From chromosomal damages evaluations it follows that with continuing recovery, reparative effect of syngeneic DNA is evaluated; at the same time, it is obvious that higher per cent of reparation (or recovery resp.) is reached in the case of the action of bi-functional alkylating agens.

If our experimental results are taken into account we may suppose that an employment of syngeneic DNA in the injured cells of the recipient occurs during the course of synthetic phase of cellular cycle. It is known from reports of numerous authors (REVELL 1953, EVANS and SCOTT 1964, LOVELESS 1966 and others) that an alkylating substance in biological systems may generally react with nucleophillic centers, as with proteins or nucleic acids respectively (centres rich of electrones). Direct alkylation of DNA is considered to be one of the main causes of induction of chromosomal aberrations by alkylating substances. In mentioned macromolecule, an alkylation of primary phosphate- and aminogroups occurs. Two processes may lead to the formation of chromosomal aberrations: deletion of alkylated bases, predominantly of 7-alkylguanine (LAWLEY and BROOKES 1963) and a cleavage of deoxyribophosphate linkages directed to a break of fundamental chain of the DNA (LETT *et al.* 1962). In the case of EMS, mostly phosphate groups (ALEXANDER 1962) are alkylated with concomitant slow re-alkylation of the bases. However, at the induction of chromosomal aberrations in cellular population, also co-participation of additional toxical effects which may influence "rejoining" system, or survival respectively, is not possible to exclude. Under presumption that *Vicia faba* metabolism has a partial ability to repair injuries caused by alkylations, we may suppose that such a process is significantly stimulated by exogenously applicated syngeneic DNA. Presented results enable conclusions with a statement that the mentioned biomacromolecule participates itself in the process of reparation, most probably during the course of own DNA synthesis.

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JANA ŠLOTOVÁ, Z. KARPFEL, DAGMAR KUBÍČKOVÁ, Biofysikální ústav ČSAV, Brno: **Účast exogenní DNK v reparačních procesech meristematických buněk *Vicia faba* poškozených monofunkčním alkylačním činidlem ethylmethan sulfonátem.** — Biol. Plant. **16** : 21—27, 1974.

V práci byl testován vliv exogenní DNK syngenního původu na průběh zotavení meristematických buněk primárního kořene *Vicia faba* po působení monofunkčního alkylačního agens — ethylmethansulfonátu (EMS). Hodnocen byl časový průběh změn mitotické aktivity studované buněčné populace a dynamika tvorby postmetafázových chromosomových aberací. Výsledky ukázaly, že syngenní DNK významně napomáhá reparaci poškozených buněk; její aplikace vyvolala zvýšený výskyt buněčného dělení již v raných intervalech zotavení, doprovázený současným snížením mikroskopicky zachytitelných chromosomálních poruch. Studium lokalisace indukovakých poškození v metafázových chromosomech upozornilo na zvýšenou sensitivitu malých chromosomů *Vicia faba* vůči EMS. Rovněž reparativní účinek syngenní DNK se projevil přednostně v téže chromosomální skupině, a to významným snížením frekvence aberací. Podrobnou klasifikací indukovaných aberací bylo stanoveno procentuální zastoupení jednotlivých druhů, které nebylo působením syngenní DNK nijak ovlivněno. Ukázalo se, že v obou případech největší převahu měly isochromatidové zlomy.