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SOME NEW DATA CONCERNING THE MUTAGENIC ACTION OF DNA

It is shown that spermatozoa of Drosophila melanogaster can transfer molecules of exogenous DNA (or their fragments) from a solution of this DNA injected into the haemocel of an adult male into the ooplasm of the egg. As a result, mutations induced in the descendants arise both in the paternal and the maternal chromosomes obtained by them. The mutagenic effect of exogenous DNA has a prolonged character inducing mutations in the descendants during many cell generations after DNA-treatment of their father. The mechanism of such a prolongation of the mutagenic action of DNA is discussed.

In our previous experiments [1, 2] on the induction of recessive lethal mutations in the 2nd chromosome of *Drosophila melanogaster* by injections of a solution of exogenous DNA into the haemocel of adult males we found that the frequency of induced lethal in the progeny which developed from eggs laid by the female fertilized by a DNA-treated male during the first three days after copulation was in most cases only slightly different from the frequency of such mutations in the progeny which developed from eggs laid later. This surprised us as it meant that DNA can induce mutations not only in the dividing premeiotic germ cells of the males but also in their completely formed ripe spermatozoa. This was later confirmed by the results of an experiment in which DNA-treated males were immediately after their first copulation separated from the females with which they have copulated, so that we could be certain that the eggs laid by the females were fertilized by spermatozoa which were in contact with exogenous DNA already being ripe. The frequency of lethals induced by DNA was here again significantly higher than in the untreated control.

In seems utterly unbelievable that very large DNA molecules present in the solution injected into the haemocel of the male can penetrate into the tightly compressed nucleus forming the head of the spermatozoan. Much more probable is that DNA molecules are adsorbed on the surface of the spermatozoan and thus are purely mechanically transported and introduced into the ooplasm of the fertilized egg where they reach both the male and female pronuclei and eventually the paternal and maternal chromosomes in them.

If this supposition is correct then exogenous DNA injected into the male should induce mutations in chromosomes received by the descendants both form their treated father and their untreated mother. In favour of this supposition speaks the experiment described in 1971 by Brackett et al. [3]; it showed that mammalian spermatozoa can introduce into the egg DNA molecules adsorbed by them from the surrounding fluid. Recently this has been confirmed by Lavitrano et al. [4] and by Siracuso et al. [5]; their experiments showed, for example, that murine spermatozoa, incubated in an isotopic buffer containing an exogenous DNA (the plasmid *pSV2 CAT*) are introduced into eggs fertilized by them. Embryos thus obtained were implanted into pseudo-pregnant females and among 250 descendants of these females about a third contained in their genomic DNA nucleotide sequences of the above-mentioned plasmid. And when

transgenic females were crossed with *CD1* males the gene *CAT* was expressed in the bones and tails of their descendants. In all, these authors had proved that such a transport of exogenous DNA into the ooplasm can be achieved by spermatozoa of mice, bulls and man; and it is quite probable that this can place in insects.

To check this hypothesis we carried out an experiment using as markers the following mutant genes located in the 2nd chromosome of *D. melanogaster* — *Curly (Cy), Bristle (Bl), Lobe*^{2} *(L^2), black (b)* and *cinnabar (cn)*. Calf thymus DNA was dissolved in saline and injected (ca. 0.07—0.09 micrograms of DNA in 0.25 ml of fluid per male) into the haemocel of heterozygous males having one of its 2nd chromosomes marked with *Cy* and *Bl* and the other with cn (*Cy Bl/cn*). These males were crossed with virgin heterozygous females having one of its 2nd chromosomes marked with *Cy* and the other with *b*(*Cy/b*). The F_1 from these crosses consisted of flies including two *Curly* classes, *Cy/cn* and *Cy/b* which can be easily distinguished one from another. Each of the F_1 *Cy Bl/b* males was individually crossed with a virgin *Cy/L^2* female and their F_2 descendants were inbred, this allowing to detect in the F_3 recessive lethal mutations which had arisen in the 2nd chromosome of their untreated grandmother (in this case no *black* flies will be present in the F_3). Likewise, each of the F_1 *Cy/cn* males was individually crossed with a virgin *Cy/L^2* female and their F_2 descendants were inbred, this allowing to detect in the F_3 recessive lethal mutations which had arise in the 2nd chromosome of their DNA-treated grandfather (in this case no *cinnabar* flies will be present in the F_3).

The results of this experiment is presented in Table 1. As shown in this table, the frequency of recessive lethal mutations in the paternal and the maternal chromosome is about the same, exceeding about ten times the frequency of such mutations which spontaneously arose in the untreated control. This allows us to be rather certain that the induced mutations arose not in ripe spermatozoa but in the chromosomes of both pronuclei (paternal and maternal) contained in the fertilized egg. (A similar experiment was performed on lethals induced in the 2nd chromosome by the synthetic polyribonucleotide poly(A, U). This experiment also showen that the mutagen can be carried by spermatozoa to the female pronucleus. The number of lethals induced in the paternal (5.73 %) and the maternal (4.50 %) chromosome of F_1 flies was nearly equal.)

However, as this experiment was carried out on a relatively modest scale this conclusion is only preliminary and needs a repetition on a larger scale.

In one of the experiments of Fahmy and Fahmy [6] in which exogenous DNA was injected into adult Drosophila males, a statistically significant increase was observed among F_1 females of the mutability of the *garnet* locus in the X chromosome received from their mother and not from the DNA-treated father. These authors assume that here the exogenous DNA injected into the father was passively transmitted by the spermatozoa to his daughters. We are inclined to regard this case as speaking for the correctness of our conclusion based on the data presented in Table 1.

Table 1

Recessive lethal mutations induced in the 2nd chromosome of Drosophila melanogaster in experiments on transportation of DNA molecules by spermatozoa into the egg

Source of chromosomes	Number of chromosomes tested	Number of induced lethals	Percentage of lethals
Paternal	204	8	3.9±1.4 and 1 miniature mutation
Maternal	178	6	3.3±1.34
Control (no treatment)	235	1	0.4±0.4

Already in our first experiments [7] on the induction of mutations in *D. melanogaster* by exogenous DNA it was found that the mutagenic action of DNA is often not immediate but considerably delayed so that many mutations in the F_1 of DNA-treated males appear as mosaic individuals. As was shown by these experiments and numerous later ones, some gene mutations induced by exogenous DNA added to the food of *D. melanogaster* larvae or injected into the haemocel of adult males appear in the F_1 as buches of mutants (this showing that the arose in premeiotic germ cells of the male) or as whole (non-mosaic) individuals but many of them, usually more than 50 per cent, appear as mosaic in which only 1/2, 1/4 or a lesser part of their body consists of mutant tissues. For example, in the F_1 of some such experiments among 13 247 flies 27 visible gene mutations were found twenty of which appeared as mosaics (chiefly 1/2 or 1/4 mosaics). This group included flies manifesting phenotypical mosaicism (e. g. with one normal and one *miniature* wings) which had mosaic gonads; and also it included some phenotypically whole mutant (non-mosaic flies which had mosaic gonads [8]). On Table 2 are shown the results of crossing such F_1 mosaic males with virgin attached-X females. Other mosaic males in this and other similar experiments gave analogous results. To the 20 mosaic flies in this experimental series a number of phenotypically mosaic flies probably should be added which gave no offspring or gave only wild-type descendants and therefore were not registered as mutants.

A preponderance of mosaic mutants over whole ones was characteristic not only of sex-linked visible mutations but also of autosomal ones and was observed in our experiments not only in the F_1 but in the F_2 and F_3 as well.

Many F_1 mosaics in which the mutant part was *ca* 1/4 of the body or less must have arisen as a result of mutation which took place two or more cell generations after the direct treatment of DNA had stopped. But the mutagenic effect of DNA may be manifested even later. We have shown in our experiments that the mutagenic effect of DNA is evident not only in the F_1 but also in the F_2 and the F_3 though in the latter it is somewhat weaker; so it is still present several dozens of cell generations after the cessation of the treatment.

In some cases the same gene mutation arose in different generations of descendants originating from a single treated male. Thus, in one of our experiments a mosaic *miniature* male was found in the F_1; this male was crossed with an attached-X female and some of their sons were *miniature* and other had wild-type wings, this being a result of mosaicism of the father's gonads. One of the wild-type sons had deformed eyes and in order to analyse this deformity the male was crossed with an attached-X female. The deformed eye proved to be non-hereditary but among the offspring of this cross a single mosaic *miniature* male again appeared from which a pure *miniature* stock was later established. A similar picture was observed in the progeny of a mosaic *rudimentary* male and *rudimentary* mosaics and whole *rudimentary* males continued to appear untill the F_6. A pedigree of this family is given in Fig.

In another of our experiments an F_1 male was a *fused* mutant. In the offspring of its wild-type male brother again a *fused* mutant was fo-

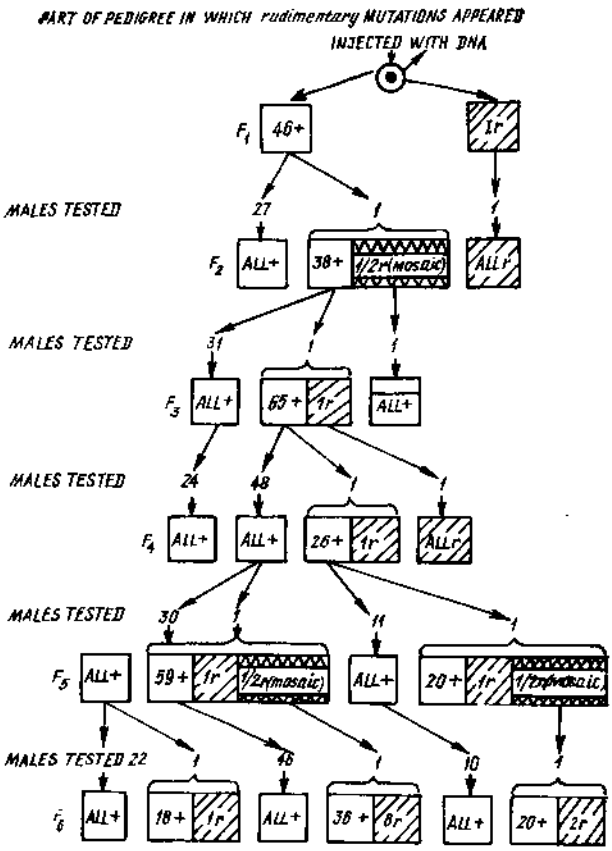
Table 2

Offspring of mosaic mutants of Drosophila melanogaster; the mutations were induced by exogenous DNA

Mutation	Phenotype of father	Offspring		
		♀♀	Mutant ♂♂	Wild-type ♂♂
Miniature	Both wings mutant	101	86	24
Miniature	One wing mutant	27	4	38
Small-wing	Both wings mutant	70	3	25

und; it was a mosaic both in respect of wings and gonads. Such cases show that treatment with exogenous DNA leads to the appearance of a transmittable unstability of certain genes.

In one case the mutant nature of an F_1 fly could be ascertained only because it was a mosaic. This fly, a male, carried a dominant autosomal mutations which we named *Beaded-crossveinless* (its phenotype reminded both the well known *Beaded* and the *crossveinless* mutations of *D. melanogaster*). When crossed with an attached-X female it gave a progeny consisting of 67 flies among which 9 females and 11 males were



Part of a pedigree in which rudimentary mutations appeared among the descendants of a mutant male in which the mutation was induced by exogenous DNA

Beaded-crossveinless. Both saxes of these mutants had underdeveloped gonads and were completely steril. Evidently, their mutant father has been a mosaic the fertility of which was due to the presence in his body of normal non-mutant tissues.

We did not study mosaicism among recessive lethal mutations induced by DNA but Mathew [9] and Khan and Alderson [10] used a genetic technique which allowed to detect in such experiments not only complete (non-mosaic) lethals but mosaic ones as well. The proportion of mosaic was here even higher than in our experiments on the induction of visible mutations. In many treated lines F_1 mosaics again produced mosaics in the F_2 and so on, up to F_5 (in the experiments of Khan and Alderson) and even to F_9 (in experiments of Mathew). The extension of the mutagenic effect of DNA to such late generations strongly supports the supposition made above about a transmittable destabilization by exogenous DNA of certain genes.

Fahmy and Fahmy [6, 11] found mosaics among visible recessive mutations and *Minutes* induced by injection into adult *D. melanogaster*

males of DNA isolated from larvae of the same species and from rat liver.

The data obtained in our previous work the induction of visible and lethal mutations in *D. melanogaster* by exogenous DNAs showed that these mutations in many important aspects closely resemble mutations induced by spontaneous insertions into the chromosomes of the recipient of mobile genetic element of several kinds [12—15] and many other. It is most unlikely that this parallelism of the peculiarities of the mutagenic effects of spontaneous insertions of mobile genetic elements and of injections of exogenous DNA is accidental. It seems much more probable that fragments of molecules of exogenous DNA act like transpositions of mobile genetic elements becoming inserted into chromosomes and selectively altering or destabilizing certain genes. If this hypothesis is correct it explains the prolonged mutagenic action of exogenous DNA. Fragments of this DNA for a time remain in a free state, like episomes, in the cells of the recipient and are thus transmitted from one cell generation to another; or they can be inserted into the DNA of chromosomes this causing gene mutations or inducing minor chromosome rearrangement this behaviour resembling that of some mobile genetic elements, e. g. *copia* (Flavell and Ish-Horowicz [16], Shiba and Saigo [17], Yamafumi et al. [18] and other authors).

The reversions to wild-type observed by us of some unstable mutations induced in Drosophila by exogenous DNA may be caused by an excision of the inserted fragment of foreign DNA or by a change of its orientation within the chromosome.

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ДЕЯКІ НОВІ ДАНІ СТОСОВНО МУТАГЕННОЇ ДІЇ ДНК

Резюме

Виявлено, що сперматозоїди самця Drosophila melanogaster здатні переносити до ооплазми запліднюючого яйця молекули екзогенної ДНК (або їхні фрагменти) з розчину цієї ДНК, що введений до гемоцелі самця; внаслідок цього індуковані ДНК мутації виникають у нащадків у хромосомах, одержаних як від батька, так і від матері. Показано, що мутагенна дія екзогенної ДНК має пролонгований характер і індукує у нащадків мутації протягом ряду клітинних поколінь. Обговорюється вірогідний механізм такої пролонгованої дії.

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