Garnett, D.J. Lovesgrove House, Aberystwyth, Wales, U.K. Detection of colour mutations in Drosophila by biochemical methods.

A sample of male $^{+}/+$ **D.melanogaster** were treated with EMS in the way described by Lewis & Bacher (DIS 43:1 93) for 15 hr and then mated with XXY strain (as Jenkins 1967). The 24hr old X_1 were scored for mutations and homogenised in batches of 25, in 1cc of 0.04M HCl. The solutions were filtered and analysed with a Unicam 1800 spectrophotometer at 200nm (5µl per 1.2cc curvette). No attempt was made to fractionate the solution and mutations throughout the body can be picked up; however, due to the fluorescent nature of the pterin eye pigments, mutations occurring elsewhere were less apparent at these higher wavelengths. It was hoped that this kind of analysis could be routinely used in this laboratory, for detecting internal changes, where any organ with distinct pigmentation is involved, e.g., eyes, malpigian tubes, etc.

Wild type - 0.323 ± 0.004

0.125M EMS treated culture - 0.356; 0.025M EMS treated culture - 0.395.

It was found in the course of these experiments that substantial discrepancies occurred when old, killed flies were used as controls for freshly prepared extracts; the solutions, made up as above, keep well at -18°C, less well at room temperature.

I thank the Wellcome Trust and Sigma Chemicals Co.

References: Stein, S.P. & E.A. Carlson 1980, DIS 55:139; Jenkins, J.B. 1967, Mut. Res. 4:90-92.

Garnett, D.J. Lovesgrove House, Aberystwyth, Wales, U.K. A new method of mutagen application to Drosophila eggs and larvae.

During the course of my work with mosaic eye mutants, I found that chemically induced mutations occurring through development can be shown by treating the embryos and larvae with the compound. Larvae are isolated and the chorion is wetted with the mutagen using an innoculating loop. The larvae are left for 5-10 minutes and are then dried with filter paper (the larvae can be preimmersed in dilute NaOCl to remove the membrane). Embryos were deposited onto microslides coated with a yeast paste hydrated with 0.25M EMS plus 0.1% sucrose and Nipagin. Eggs are counted by transilluminating the slides, so that the percentage survival can be calculated, giving some idea of the toxicity and the mutagenicity of the chemical. The embryos and the larvae are left at 25°C to develop and are scored for mutations 24 hrs after emergence. If the eggs are all deposited in a short time (Gupta 1980, DIS 55:152), the dose (concentration x time) to each will be constant.

It was also shown that insoluble powders could be tested by working into a yeast paste, 1% sucrose, on which the adult males feed for 24 hrs.

Gazaryan, K.G., S.D. Nabirochkin and E.N. Shibanova. Institute of Molecular Genetics, USSR Academy of Sciences, Moscow State University, USSR. Induction with high frequency of site-specific visible mutations in the MR-strain of **D.melanogaster** by DNA injected into the polar plasm of early embryos.

In an earlier study we obtained the eye-deformed **(edf)** mutation, an abnormal development of the eye-antennal disk, by introducing the Rous sarcoma virus (RSV) into **D.melanogaster** eggs (Gazaryan et al. 1981, 1982). RSV DNA cloned in pBR322 (pPrC11, see Ambartzumian et al. 1982) rendered similar mutagenic effect (Gazaryan et al. 1984). Neither homologous DNA, nor pBR322 caused visible mutations with such a frequency (1-2 mutants among ∿ 200 flies of F_2 progeny of each of 1 to 5 injected embryos (F_0). At that time we were dealing with the wild-type Oregon R stock of **D.melanogaster**. In the present study we used **D.melanogaster** (T-007) (MR-strain) for similar experiments. The following DNAs were introduced into the polar zone of eggs at stages 7 to 8 (70-80 min. p.o. at 25°C): pPrC11 plasmid (see above), pBR322, **D.melanogaster** DNA, rat liver DNA. To activate the MR-factor, the males of T-007 strain were crossed with wild-type Oregon R females. The results are listed in the Tables.

The most important thing is that the introduction of pPrC11 containing RSV DNA insert into the polar plasm of the MR-strain embryos causes the mutation in one locus with an extraordinarily high frequency: in up to 50% of the injected embryos. The mutation alters the development of eye facets (about 1/3 of the eye facets are fused) and has been denoted fe^{m} (fused eye, moderate). Apart from this,

Table 1. Mutant lines isolated in experiments on injection of cloned RSV DNA (plasmid pPrC11) into polar plasm of MR-strain (T-007) embryos with repressed and activated MR-factor.

Exp No	recipient line	material injected	Number of embryos injected	F_0 flies survived	mutant lines isolated	mutations
1	T-007	pPrC11	3050	77	5	Or (Orange eyes); dominant, x-chromosome
					2 (9.1%)	fe^m (fused eyes, moderate) recessive, 2nd chromosome
-"-	-"-	saline	2230	83	0	--
2	T-007/ Oregon R	pPrC11	560	82	39	fe^m
					2	Or
					1 (50%)	white
3	-"-	-"-	460	37	16	fe^m
					1	fe^w (fused eyes, weak)
					(45%)	recessive, 2nd chromosome
4	-"-	Oregon R DNA	800	78	11	fe^w
					2	white
					1 (20%)	Or
5	-"-	Rat liver DNA	840	75	10	fe^w
					5	fe^m
					2 (20%)	w
6	-"-	pBR322	830	80	11	fe^w
					2	Or
					(∿ 15%)	
-"-	-"-	saline	440	75	0	--

the Orange eyes (**Or**) and white mutations occur with a considerably lower frequency (see Table 1). A test for allelism has shown independently obtained fe^m mutations to belong to one complementation group. The injection of rat DNA, pBR322 and homologous DNA into the eggs caused the same three mutations, but the **fe** mutation occurred far less frequently and showed a lower expressivity (about 5-10% of facets are fused), so this phenotype was denoted fe^w (weak expression) to distinguish them from fe^m (moderate) phenotype caused by pPbC11. It is interesting that the same mutations (and **white** among them) occur in homozygous T-007 stock embryos where the MR-factor is repressed (see e.g., Bregliano & Kidwell 1983) but the frequency of the **fe** mutation is no higher than that of the other two. It is very important that in the T-007/Oregon R stock, where the MR-factor is active, no mutations are observed at the said loci in our samples if no DNA is introduced (saline is injected instead), but the mutations are there (the white among them) if any of the above-mentioned DNAs is injected. This means that not only the **fe** locus, which is here described for the first time as a potentially unstable one in the MR-strain, but also white, already known to be unstable in this system (Colins & Rubin 1982) show a higher mutation frequency in the presence of foreign DNA than under the action of the MR-factor alone (in the control series). Unlike the **fe** locus, white do not distinguish between DNAs of different origin, although one cannot be sure that such distinctions might not transpire if a greater number of strains are analyzed. The **fe** locus behaves in a different manner: (1) its mutability varies depending on whether oncogenic or other DNA is used; (2) activation of the MR-factor leads to a dramatic increase of its mutability, although the mechanism of this increase is still obscure.

Some of the mutant lines obtained in our previous works appeared unstable (Gazaryan et al. 1981, 1984). So, we checked the stability of phenotypic expression of 16 arbitrarily selected (from over 100 obtained) fe^m mutant lines. Up to 5th generation no changes were observed. Then, between 5th and 7th generations the phenotype of the flies in 7 lines was altered, reversed to the normal, or acquired new ab-

Table 2. Secondary mutations arose as a "burst of instability" in 5th-7th generation flies of a fe^m line.* (r = recessive; d = dominant)

Mutation	Reduced area of the facets (%)	other changes	inheritans, chromosoma
fe^w (fused eye, weak)	5-10	-	r, 2nd
edf (eye deformed)**	15-20	cuticular outgrowths on altered part of eyes	r, 2nd
Fe^s (fused eye, strong)	50-60	-	d, 2nd
Pd (Palp duplication)	70-80	Palp duplication	d, 2nd
Ce (Changed eye)†	70-80	Facts on cuticular outgrowths	?
Ad (Antennal dupl.)†	90-100	Antennal duplication	?
Od (Ocular dupl.)	90-100	Mini eye in altered region	d, 2nd
Er (Eye reduced)**	100	-	d, 2nd
Cw (changed wings)	-	Local thickening of wing veins	d, 3rd
Curly			

* In ∿ 90% of the same generation flies fe^m mutation reversed to normal.
**For more detailed characteristics see Gazaryan et al. 1981, 1982.
† the line is lost.

normalities (secondary mutations). In 3 lines we observed a kind of general instability, the "burst" of phenotypic changes: about 90% of the flies reversed to normal phenotype, about 5% maintained **fe^m** appearance and about 5% gave rise to the set of new mutations (some of them maintaining the **fe^m** together with new ones). Six out of nine secondary mutations were the same type as **fe**, e.g., led to abnormal development of the eye-antennal disk derivatives. They are characterized by more extensive fusion of facets (up to 100%) and in addition in some of them we found duplications of some of eye-antennal structures (Table 2). So, we have at present in total nine mutations in presumably one gigant locus which alter this disk development. They represent a row with gradual increase of the effect (facet fusing, mainly). Only three of them, those with weaker effects (**fe^m**, **fe^w**, edf) are recessive and arose as a consequence of the primary effect of the injected virus (Gazaryan et al. 1981, 1984) or DNA (see Table 1). The secondary mutations more strongly affect the eye-antennal disc development and most of them represent dominant mutations (Table 2).

We attempted to find the RSV-specific sequence in the DNA of the mutants. The result of in situ hybridization of ^{32}P DNA of RSV with polytene chromosomes and of Southern blot-hybridization with DNA of **fe^m** mutants were negative. However, dot-hybridization revealed in some of mutant lines (mainly in those which exhibited instability) some amount (0.2-0.4 copies per haploid drosophila genome) of RSV-specific sequence.

How can foreign DNAs specifically induce mutations (see also Gershenson et al. 1971) and what is the reason of the higher effectivity of oncogenic virus DNA?

The instability of mutations strongly suggests that they are insertional mutations. Two possibilities can be considered: (1) exogenous DNA is inserted into the loci concerned; (2) it binds proteins (e.g., repressors) mobilizing genomic IS-elements (transposons). In situ hybridization of labeled Dm5002 plasmid containing **copia** element (Dunsmuir et al. 1980) with polytene chromosomes shows that in the mutants described here the sites of **copia** localization are changed compared with the control larvae. This observation suggests that the mobilization of endogeneous elements is actually induced by the DNA injected in embryo.

References: Gazaryan, K.G., A.K. Shahbazyan, N.S. Neznanov, S.G. Smirnova, F.L. Kisselev & A.G. Tatosyan 1981, Dokl. Acad. Nauk SSSR (USSR) 258: 1224-1227; Gazaryan, K.G., A.K. Shahbazyan, N.Yu. Sakharova & S.G. Smirnova 1982, DIS 58:64-65; Ambartzumian, N.S., A.G. Tatosyan & G.N. Yenikolopov 1982, Molec.Biol. (USSR) 16:1183-1188; Gazaryan, N.G., S.D. Nabirochkin, A.K. Shahbazyan, E.N. Shibanova, T.I. Tichonenko, L.V. Gening & V.A. Goltzov 1984, Genetica (USSR), 20:1237-1244; Bregliano, G.C. & M.G. Kidwell 1983, in: Mobile Genetic Elements (ed. J.A. Shapiro) Acad. Press, N.Y., pp. 329-361; Colins, M. & G.M. Rubin 1982, Cell 30:71-79; Gershenson, S.M., Y.N. Alexandrov & S.S. Maluta 1971, Mutation Res. 11:163-173; Dunsmuir, B., W.Y. Brorein, M.A. Simon & G.M. Rubin 1980, Cell 21: 575-579.