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Jean-Claude Bregliano, Anne Laurençon, Fabienne Degroote. Evidence for an inducible repair-recombination system in the female germ line of *Drosophila melanogaster*. I. Induction by inhibitors of nucleotide synthesis and by gamma rays.. *Genetics*, 1995, 141 (2), pp.571-8. hal-03079248

HAL Id: hal-03079248

<https://hal.science/hal-03079248>

Submitted on 17 Dec 2020

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Evidence for an Inducible Repair-Recombination System in the Female Germ Line of *Drosophila melanogaster*. I. Induction by Inhibitors of Nucleotide Synthesis and by Gamma Rays

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Manuscript received July 28, 1994

Accepted for publication June 30, 1995

ABSTRACT

In the I-R system of hybrid dysgenesis in *Drosophila melanogaster*, the transposition frequency of *I* factor, a LINE element-like retrotransposon, is regulated by the reactivity level of the R mother. This reactivity is a cellular state maternally inherited but chromosomally determined, which has been shown to undergo heritable, cumulative and reversible changes with aging and some environmental conditions. We propose the hypothesis that this reactivity level is one manifestation of an inducible repair-recombination system whose biological role might be analogous to the SOS response in bacteria. In this paper, we show that inhibitors of DNA synthesis and gamma rays enhance the reactivity level in a very similar way. This enhancement is heritable, cumulative and reversible.

In *Drosophila melanogaster*, two main systems of hybrid dysgenesis have been described, referred to as I-R and P-M (reviewed in BREGLIANO and KIDWELL 1983; ENGELS 1989; FINNEGAN 1989; BUCHETON 1990). In both systems, the *melanogaster* species divides into two main categories of interacting strains. One of them bears a given family of mobile element (*I* or *P*), the other is devoid of that element, at least in its active form. In the I-R system, the two classes of strains are denoted "inducer" and "reactive" (PICARD and L'HERITIER 1971; PICARD *et al.* 1972). The inducer strains carry five to 15 copies of *I* elements per haploid genome dispersed on their chromosome arms (PELISSON and BREGLIANO 1987); some of them, referred to as *I* factors, are active for transposition. The reactive strains do not have any *I* elements on their chromosome arms. Both categories of strain bear a similar pattern of defective *I* elements in the pericentromeric β heterochromatin (VAURY *et al.* 1990).

When a cross is performed between a reactive female and an inducer male, the F₁ daughters, known as SF females, are more or less sterile; they lay a normal number of eggs but the hatching percentage is abnormally low. This sterility is maternally determined; it is independent of the males with which the SF females are mated. The F₁ males show normal fertility, as do the progeny of both sexes in the reciprocal cross. The surviving progeny of SF females exhibit a high level of mutations, chromosomal rearrangements and chromosome losses, (PICARD *et al.* 1978; PROUST and PRUDHOMMEAU 1982; PROUST *et al.* 1992). In most nonhatching

eggs, the development is stopped very early, between the first and the third division and large numbers of chromosomal fragments and bridges are observed (LAVIGE and LECHER 1982; LAVIGE 1986). These features are very similar to those observed after irradiation of mature oocytes with X-rays (TRAUT and SCHMIDT 1967). Transposition of *I* factor is known to occur at high frequency only in the germ line of SF females (PICARD 1976); therefore, the most likely explanation of all available data on SF sterility is that it is a direct consequence of this transposition, either through complete transposition events leading to chromosome exchanges and sister chromatid union or through incomplete transpositions leading to unrepaired chromatid breaks. Most of the mutations and chromosome losses appear as isolated events (PICARD *et al.* 1978); this means that the transposition of *I* factor rarely occurs at premeiotic stages. The fact that it does not occur at all in spermatogenesis suggests that it may be linked in some way to a specific function of oogenesis.

As in the P-M system, a high frequency of transposition appears only when males with active transposable elements are crossed with females that are devoid of them. This is called the permissive cross. The reciprocal cross does not allow a high frequency of transposition. In the P-M system, it has been demonstrated that the main cause of this limitation is the presence, in the *P* flies oocytes, of a repressor encoded by *P* elements (RIO 1990; LEMAITRE *et al.* 1993). The same situation probably occurs in the I-R system (PELISSON and BREGLIANO 1987; MCLEAN *et al.* 1993), although the molecular identification of the *I* repressor is not completed yet.

In the I-R system, the frequency of transposition in the permissive cross may vary within a wide range, de-

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pending on the parental stocks (in particular on the reactive one) used in the cross. There is a quantitative continuum of strength variation within the reactive category, the strength being measured by the percentage of nonhatching eggs laid by the SF females. When they originate from the weakest reactive mothers, the SF females exhibit normal fertility. The transposition of *I* factors does occur in their germ line, but is not frequent enough to induce lethality. Conversely, when they originate from the strongest reactive strains, SF females lay exclusively nonhatching eggs, at least when they are young. All intermediate strengths occur. Thus, we can recognize a wide range of so-called reactivity levels that determine the frequency of transposition of *I* factors in the permissive cross.

In the past, there has been a great deal of research aimed at understanding the genetic determinism of the levels of reactivity; the results show a complex mixture of chromosomal and maternal inheritance (reviewed in BREGLIANO and KIDWELL 1983). Crosses between strong and weak reactive strains show that the reactivity level is inherited mainly maternally from one generation to the next. However, over several generations, the chromosomes appear to be the major controlling determinants (BUCHETON and PICARD 1978).

Nongenetic factors can modify the reactivity level, and these modifications are heritable, cumulative, and reversible. One of these factors is aging and its effect can be summarized as follows. When a strong or a medium reactive stock is bred with short generations, *i.e.*, each generation being recovered from young mothers, the level of reactivity remains stable. If the same stock is bred with long generations (old mothers), reactivity progressively decreases and may reach a very low value. This new level is stable as long as the long generation pattern is maintained; if the stock is put back onto a short generation pattern, the level of reactivity rises again and progressively reaches the original level. But, whichever the direction, the complete shift requires several generations. Reactivity is also decreased when oogenesis takes place at 29° (BUCHETON 1978, 1979).

All available data on the levels of reactivity lead to the conclusion that they are determined by a cellular state of the mature oocytes laid by the reactive females; therefore oogenesis plays a key role in their control. But hitherto, the biological function underlying this cellular state, as well as the molecular basis of its puzzling inheritance, has remained a mystery. Recent work, using *I-lacZ* constructs, has demonstrated that the effect of reactivity levels is to control expression of *I* factor, probably by regulating transcription (LACHAUME and PINON 1993).

Several features of reactivity, especially the changes induced by environmental factors, have led us to believe that it might be one manifestation of a stress-response system controlling DNA stability. According to this view, its biological role might be comparable with that of the

SOS response in bacteria. In *Escherichia coli*, the SOS network plays a major part in the control of mutation and recombination frequency in response to mutagenic agents, inhibitors of DNA replication, and some abnormal physiological conditions (WALKER 1985; DEVORET 1993; F. TADDEI, I. MATIC and M. RADMAN, unpublished observations). In plants, the existence of such inducible repair mechanisms has been proposed (BURR and BURR 1988). In animals some indirect evidence has been described for cultured cells (SARASIN and BENOIT 1986; WILLIAMS *et al.* 1989). More recently BAKALKIN *et al.* (1994) showed that the P53 protein, known to be inducible by mutagenic agents, binds single-stranded DNA ends and promotes DNA strand transfer reactions, suggesting that it might play a direct role in DNA repair. As far as we know, no direct proof of the existence of inducible repair activities in higher organisms is available yet.

Our aim was to investigate this putative analogy between reactivity and SOS response. For this purpose we addressed the two following questions: is reactivity level enhanced by the same agents that turn on the SOS network, namely inhibitors of DNA synthesis and physical mutagenic agents and is reactivity level related to repair-recombination efficiency?

In this paper, we describe experiments carried out to address the first question. We show that reactivity is enhanced by inhibitors of nucleotide synthesis and by gamma rays. As in the case of aging and thermal treatments, the effect is heritable, cumulative and reversible. In another paper, we describe a first set of data dealing with the second question (LAURENÇON and BREGLIANO 1995).

MATERIALS AND METHODS

Strains: The following *D. melanogaster* strains were employed: *ebony*, a medium reactive stock kept in our laboratory for a long time; *est28*, a medium reactive line derived from *ebony* 20 years ago; *ew2*, a medium reactive isofemale line recently derived from *ebony*; Paris, a weakly reactive strain caught in that city in 1952; and B2' and Canton-S are standard inducer stocks. Additional information about the genetic markers can be found in LINDSLEY and ZIMM (1992). All stocks used in this work are devoid of *P* elements.

Breeding conditions: Because reactivity is very sensitive to breeding conditions, all work on this phenomenon requires accurate control of several parameters. All stocks were maintained with short generations, *i.e.*, each generation is recovered from very young parents. Flies were reared on the axenic food described by DAVID (1959), under uncrowded conditions at 20 ± 0.5°, with a normal light-dark cycle.

Measure of fecundity and fertility of reactive females: Females were mated in vials with normal food and transferred to fresh food every 24 hr. Two days after egg laying, the food was carefully taken out of the vial, hatched and nonhatched eggs were scored; this enabled fecundity (total number of eggs) and fertility (hatching percentage) to be measured. Then the food was put back in the vial to recover adult flies.

Measure of the levels of reactivity: The same protocol was used, except that in this case the reactive females were always

mated with inducer males of a standard stock, either Canton-S or B2'. Their 24-hr-old SF daughters were put on fresh food with sib males and allowed to lay eggs during another 24 hr. Then they were discarded and 2 days later the percentage of nonhatching eggs was scored.

Inhibitors of nucleotide synthesis: Methotrexate (Sigma) is known to block dTMP synthesis by inhibiting dihydrofolate reductase (CARPENTER 1974) and therefore blocks DNA synthesis; 6-azauracil (Sigma) inhibits the orotidine-5'-phosphatase-decarboxylase and leads to arrest of both RNA and DNA synthesis (JONES 1980). Both were applied to the flies by feeding. We put in a vial devoid of food a piece of filter paper soaked with a solution of sucrose 0.25 M, with 0.5 ml of the solution of the drug to be tested. Five day-old females were placed in the vial for 24 hr, then transferred onto usual food with males. Control females underwent the same procedure with water and sucrose only.

Irradiation with gamma rays: Some experiments were performed with a ^{137}Cs source; others were performed with a ^{60}Co source. Dose rates were 4 rad/sec and 3.4 rad/sec, respectively. The flies treated were either 24-hr-old virgin females or 96-hr-old pupae.

Statistical procedures: ANOVA statistical test on percentages was performed after angular transformation. To estimate the effects of irradiation on the reactivity levels, the following formula was used: $E_x = (R_x - R_c)/(1 - R_c)$, where E_x is the estimated effect of one or several irradiations; R_c the mean reactivity level of the control flies and R_x the mean reactivity level of the treated flies. The statistical comparison between the effect of successive irradiations was achieved by the mathematical procedure described in PROUST *et al.* (1972).

RESULTS

Inhibitors of nucleotide synthesis enhance the reactivity levels: At the end of the 1970s, it had been supposed that reactivity was due to a symbiont (bacteria, mycoplasma or virus). To test this hypothesis, assays were performed with several antibiotics and other biochemical inhibitors. Unexpectedly, it appeared that fluorodesoxyuridine (Fudr) had an enhancing effect on the levels of reactivity (A. BUCHETON, personal communication). No interpretation was proposed at that time. To gain further insight, the effects of other inhibitors of nucleotide synthesis were tested.

Six to 10 sets of six reactive females (5 days old) were treated as described in MATERIALS AND METHODS, then mated with B2' inducer males and transferred to new vials every day during 3 weeks. The changes in the level of reactivity over time were measured by the percentage of nonhatched eggs laid by the SF progeny from successive batches of laying. The data obtained with three concentrations of methotrexate on the medium reactive st28 strain are shown on Figure 1 and ANOVA statistical analysis is presented in Table 1. At 1.25 and 1 mg/ml the effect on the reactivity level is strong until the 11th day after treatment, then it decreases. The highest dose does not exhibit a stronger effect than the 1 mg/ml dose. With the lowest dose (0.75 mg/ml), there is only a slight but significant increasing effect during the first days followed by a slight decreasing one. The great difference between the responses to 0.75 mg/

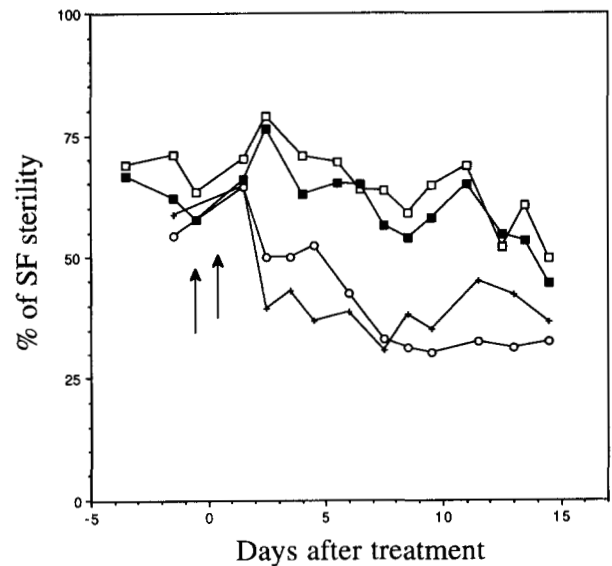


FIGURE 1.—Changes in reactivity level of st28 females after treatment with various concentrations of methotrexate: 0.75 mg/ml (○), 1 mg/ml (□) and 1.25 mg/ml (■), compared with the control (x). Females were 5 days old at the time of treatment. Arrows delimit the period of treatment.

ml and to 1 mg/ml indicates a threshold effect. The same st28 strain was treated with two doses of 6-azauracil, 5mg/ml and 10 mg/ml, the responses are similar to that observed with 1 and 1.25 mg/ml of methotrexate (Table 1). Doses <5 mg/ml have not been tried.

Several other drugs were assayed for their effect on reactivity, including erythromycin, paromomycin, DDT, malathion, sulfanilamid and azaserine (J.-C. BREGLIANO and F. DEGROOTE, unpublished data). They were applied according to various procedures, either to test for their effect on reactivity level (as above) or to test for a difference in sensitivity between strong and weak isogenic strains. All results were negative except with sulfanilamid and azaserine, which exhibit similar enhancing effects as methotrexate and 6-azauracil. Thus, among all drugs tested the only ones able to enhance reactivity are inhibitors of nucleotide synthesis.

The kinetics of the response probably means that a wide range of different egg-chamber stages are able to respond to the treatment and that the enhancement of the biochemical function responsible for the reactivity levels may last 2 weeks in the most immature oocytes.

Gamma rays display the same effects as inhibitors of nucleotide synthesis: Although the effect of chemical inhibitors is of interest, these drugs do present some drawbacks. First, it is not possible to monitor the quantity truly absorbed by the flies; it is well known that for some drugs there may be high interindividual variability. Even when the treatment is applied by injection instead of feeding, the real dose reaching the oocytes is unknown. Second, all the inhibitors used above have toxic secondary effects that may cast doubt on the significance of the data. With physical mutagenic agents

TABLE 1
Effects of inhibitors of nucleotide synthesis on the reactivity level of adult females

Inhibitor	Compared series	Days after treatment			
		Days 2 to 5		Days 6 to 9	
		d.f.	<i>P</i>	d.f.	<i>P</i>
Methotrexate	Control and 0.75 mg/ml	70	0.001	52	0.218
	1 mg/ml and 1.25 mg/ml	46	0.377	34	0.182
	Control, 1 mg/ml, and 1.25 mg/ml	77	<0.001	57	<0.001
	0.75 mg/ml, 1 mg/ml and 1.25 mg/ml	85	<0.001	63	<0.001
6-azauracil	5 mg/ml and 10 mg/ml	77	0.481	58	0.217
	Control, 5 mg/ml and 10 mg/ml	108	<0.001	81	<0.001

The ANOVA statistical test is applied on data plotted on Figure 1 and on 6-azauracil treatment. It shows that the differences between control and flies treated with the highest doses are strongly significant; the difference between 0.75 mg/ml methotrexate and the control is significant only until day 5; with both inhibitors, the differences between the two highest doses are not significant. d.f., degree of freedom; *P*, probability that the difference between the compared series is due to chance.

the doses received by the oocytes are known and the only effects are DNA damage, mainly single- and double-strand breakage. There is no secondary toxicity, except at very high doses, which were not used in this work.

The experimental procedure was the same as with drugs except that the flies were treated with γ rays 24 hr after eclosion instead of 5 days after; they were subsequently mated with inducer males of the Canton-S stock. In a first experiment performed with females of the *ebony* stock, two doses were applied: 36 and 48 Gy. For each dose and for the control, five sets of five females each were used. There is a threshold effect: the 36 Gy dose gives only a slight response whereas the 48 Gy dose gives a strong response (Figure 2 and Table 2). The kinetics of the response are similar to those displayed by inhibitors of nucleotide synthesis.

In a second experiment, performed with a subline of the same *ebony* stock, we used three doses: 48, 55 and 72 Gy; with each dose, five sets of five females were irradiated. The effects of the three doses are not statistically different (Table 2). Within the period from day 6 to 20, the variance of the 48 Gy series is higher than that of the control (1.8-fold), this may indicate an important interindividual variability in the response to gamma rays. Data provided by the higher dose exhibit an even stronger variance (3.7-fold that of the control); but in this case, most of the variability may be due to the strong disturbance of oogenesis by high level of DNA damage. In this experiment, fecundity and fertility of treated and control females were measured. The number of eggs laid by the former is greatly reduced within the first week with 72 Gy treatment and not at all affected with 48 Gy (Table 3). Mature oocytes are the most sensi-

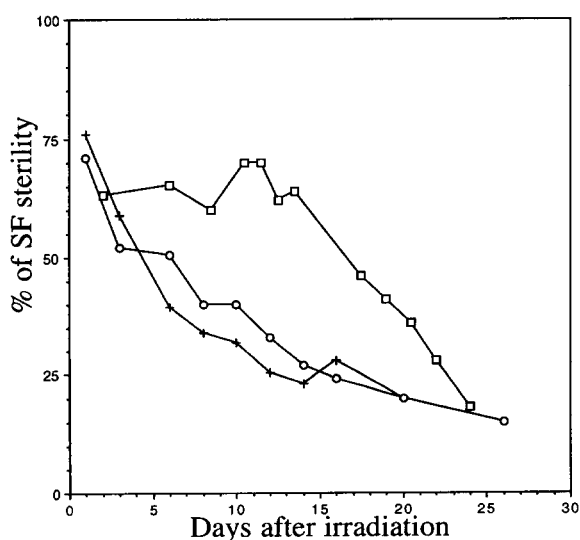


FIGURE 2.—Changes in reactivity level of females of the *ebony* stock after irradiation with a 36 Gy dose (O) and a 48 Gy dose (□), compared with the control (x). Females were 24 hr old at the time of irradiation. Statistical analysis of these data is presented in Table 2.

TABLE 2
Effect of gamma rays on the reactivity level of adult females

Experiment	Compared series	d.f.	<i>P</i>
I	control and 36 Gy ^a	48	0.035
	control and 36 Gy	68	0.140
	control and 48 Gy	69	<0.001
	36 Gy and 48 Gy	69	<0.001
II	control and 48 Gy	61	<0.001
	control and 55 Gy	61	<0.001
	control and 72 Gy	53	0.001
	48 Gy, 55 Gy and 72 Gy	87	0.649

The ANOVA statistical test is applied on data provided by two separate experiments; the data of experiment I are those plotted on Figure 2. The period taken into account runs from day 6 to 20 after irradiation. d.f., degree of freedom; *P*, probability that the difference between the compared series is due to chance. Experiment II shows that the effect of doses from 48 Gy upward is not different.

^a Period analyzed is from day 6 to 16. With the 36 Gy dose, only this period shows a significant difference with the control (5% level).

TABLE 3
Number of eggs laid by control and irradiated females of the *ebony* stock

Series	Days after irradiation										Total eggs	Percent of control
	2 + 3	4	5	6	7	8	9	10 + 11	12	13		
<i>e</i> control	26	16.9	13.6	12.5	11.2	6.2	8.5	15.6	6.9	7.9	125.3	100
<i>e</i> 48 Gy	20.4	18.2	14.7	12.6	15.4	8.8	8.2	12.4	6.5	6.2	123.4	98.5
<i>e</i> 72 Gy	28	0.3	1.16	6.1	9.2	6.2	3.2	14.6	6.7	7.4	82.9	66.2

Numbers of eggs are mean numbers for a female; the number of females is 25 for each series.

tive to DNA damage, the most immature ones are the least sensitive (Figure 3); this is in agreement with results of other authors (TRAUT and SCHMIDT 1967; SANKARANARAYANAN and SOBELS 1976). With the 48 Gy treatment, the hatching percentage nearly reaches the control value from the 11th day on (Figure 3), when the enhancement of reactivity level just reaches its maximum value (Figure 2). This result, together with the lack of fecundity difference between the control and the 48 Gy series, precludes the possibility that the enhancement of reactivity might be due to a selective bias (by selection of the strongest reactive offspring) rather than to an induction process.

In this experiment we used another control. To verify that the effect of γ rays was actually an enhancement of reactivity and not an heritable effect of irradiation *per se*, we crossed treated females (48 Gy) with reactive males and measured the hatching percentage of the eggs laid by their daughters. As expected, the results were identical to those provided by control females (data not shown).

The *ebony* stock is a medium reactive one, and it was of interest to know whether weakly and strongly reactive strains are inducible too. With regard to strongly reactive strains, we will see in a subsequent paper that the

situation may be rather complex (LAURENÇON and BREGLIANO 1995). In this report we only address the question for weakly reactive strains. Figure 4 shows the results obtained with a 48 Gy irradiation of adult females of the very weak reactive strain Paris. It appears that enhancement, although significant, is very low; two other weakly reactive stocks, HJ30 and *ery*, gave the same result (data not shown). This may mean either that all weakly reactive stocks are noninducible or that they are less sensitive and would need higher doses to be induced. Further investigations have not been made because, as seen above, higher doses generate too many anomalies on the oogenesis process which obscure the data.

Cumulative effect of gamma rays: With the *aw2* line, which is somewhat more weakly reactive than the *ebony* stock, we tested whether the effect of irradiation was cumulative over generations, as is the case with aging and temperature treatment. It is experimentally difficult to study the cumulative effect of adult treatments, because of the strong effects of age on reactivity. To overcome, at least in part, the above difficulty we irradi-

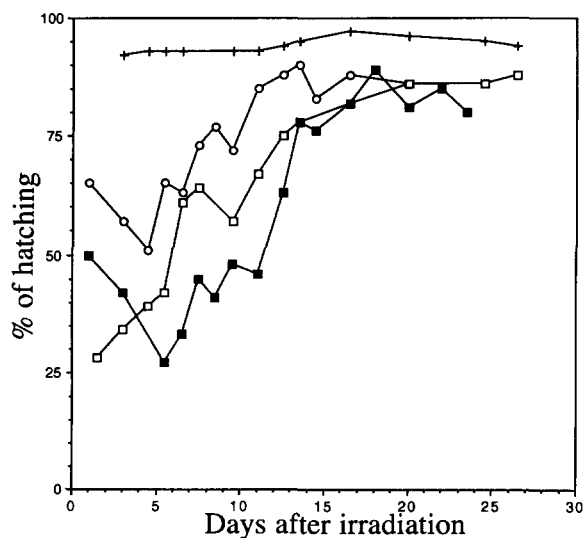


FIGURE 3.—Fertility of females of the subline of the *ebony* stock after irradiation with three doses of γ rays: 48 Gy (O), 55 Gy (□) and 72 Gy (■), compared with the control (x).

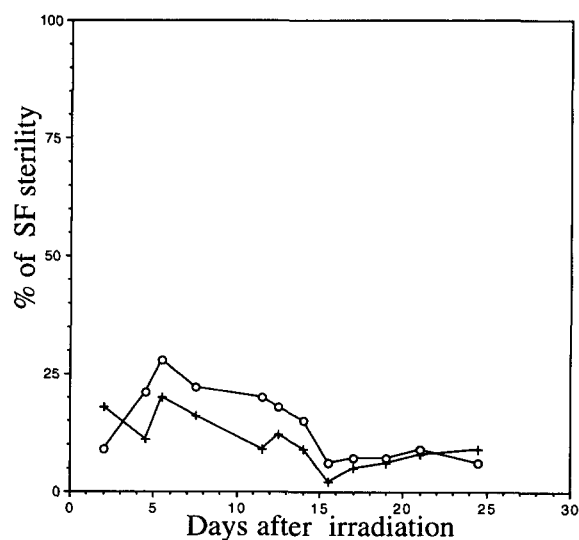


FIGURE 4.—Reactivity level of females of the weak reactive Paris stock after a 48 Gy irradiation (O). The control is shown (x). A test of fit for the extreme value distribution of binary data indicates that the probability to get by chance 10 successive points of the treated series (of 12) upper than the control series is only 0.004.

TABLE 4
Reactivity level of the *ew2* stock after several irradiations in successive generations

Days ^a	Second generation			Third generation			Fourth generation		
	C	Irr.2	SL	C	Irr.3	SL	C	Irr.4	SL
1	46.1	54.8	NS	39.6	51.4	NS	26.5	52.0	**
2	30.0	47.8	*	30.1	51.0	**	24.0	40.0	**
3	24.6	48.5	**	24.4	49.7	**	12.1	34.8	**
4	21.8	49.4	*	20.2	47.4	**	10.9	34.8	**
5	—	—		16.6	37.4	*	8.7	32.3	**

C, control flies; Irr.2, 3 and 4, flies on lines irradiated at 2, 3 or 4 successive generations (for more details see text); SL, level of significance for the difference between control flies and Irr.2, Irr.3 or Irr.4 flies; * $P < 0.05$; ** $P < 0.01$; NS, not significant. The difference between control and onefold irradiation is not presented, only a few couples of points are significantly different.

^a Age of reactive females.

ated pupae. Preliminary results showed that 96-hr-old pupae irradiated with 40 Gy develop quite normally and that adults exhibit an enhanced level of reactivity from the first days of their life. However, young pupae are far more sensitive to gamma rays than adults, so that it is not possible to use doses higher than 40 Gy, therefore it is not surprising that in this experiment the enhancement of reactivity level is rather weak.

The experiment was performed over four successive generations. For each generation, the flies tested for the reactivity level were: unirradiated flies (control), onefold irradiated flies at the given generation, and x -fold irradiated lines, x being the number of the given generation. Each generation was recovered from 4-day-old mothers; therefore there was a slight aging effect that tended to decrease the reactivity level, as observed with the control flies (Table 4). Measurement of reactivity was performed with six sets of four reactive females, during the first 5 days of their life.

The effect of irradiation is clearly cumulative over the two first generations, then the reactivity reaches an upper limit. The third and the fourth irradiations have only a slight enhancing effect (Tables 4 and 5). It cannot be excluded that this upper limit is an artifact due to the method of measuring reactivity levels. The enhancement is reversible as shown on Figure 5 for the threefold irradiated line.

DISCUSSION

The first important point to emphasize is the similarity between the results described here and the modality of induction of the SOS response in *E. coli*. It is well known that physical mutagenic agents and inhibitors of DNA replication, such as methotrexate, are powerful inducers of the SOS network (WALKER 1985). Our results show that these two kinds of agents not only cause enhancement of the reactivity level, but they do it in a very similar way. They both exhibit a threshold effect and similar kinetics of response. These analogies, between otherwise very different agents, strongly support the hypothesis that they act on the same cellular mecha-

nism. In addition, these data suggest that the same inducing signal might be involved in both *Drosophila* and *E. coli*. The most important inducing signal in bacteria is thought to be single strand DNA (WALKER 1985; DEVORET 1993).

We saw above that the data obtained with gamma rays cannot be explained by a selective bias favoring the strongest reactive offspring. For methotrexate, although accurate checking of fecundity and fertility was not done in that case, there was no discrepancy between the number of SF daughters provided by the control and the treated flies. Thus the selective bias can also be ruled out for this drug.

It appears that the relationship between doses of γ rays and levels of reactivity is not simple. For a medium reactive strain, the response to different doses of enhancing agents follows a multihit pattern (Figures 1 and 2; Tables 1 and 2). In addition, for a given dose, the response of stocks which display different levels of reactivity is also complex (Figures 2 and 4). It is not possible to know whether these features are actually due to the primary response to agents or to a complex

TABLE 5
Comparison of the effect of single to several successive irradiations on the reactivity level of the *ew2* stock

Days ^a	Number of successive irradiations		
	2	3	4
1	14.40	25.45	25.90*
2	5.35	15.04	25.30*
3	16.55	23.20	25.80**
4	21.24	18.22	18.27*
5	—	20.09	17.34*

The data are from the same experiment as in Table 4; they are presented as the difference (in percent) between the effect of several successive irradiations and the effect of a single irradiation (for the calculation of effect, see MATERIALS AND METHODS). * $P < 0.05$; ** $P < 0.01$. Taken separately for each day, the effect increase is not significant for two and three successive irradiations.

^a Age of reactive females.

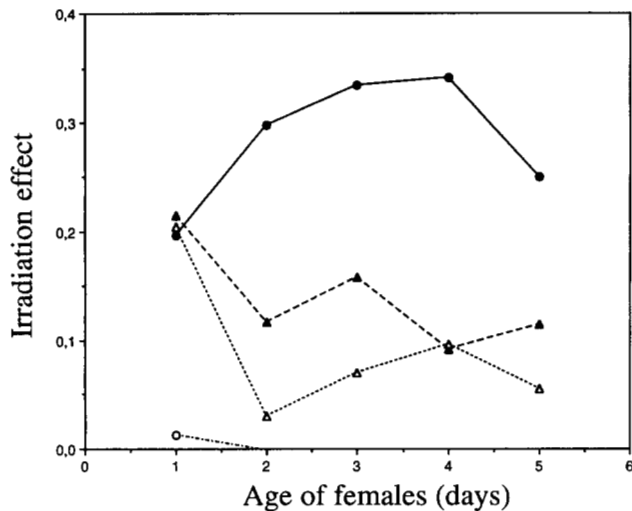


FIGURE 5.—Decrease of enhancing effect over generations after arrest of repeated irradiations. Effect of threefold successive irradiations (●), remaining effect after one generation without treatment (▲), after two generations (△) and after three generations (○). For the calculation of effect, see MATERIAL AND METHODS.

succession of events which certainly occur between this primary response and the observed SF sterility. This will be clarified only when we have a direct biochemical test for reactivity.

As said in the Introduction, reactivity levels vary widely between stocks. The simplest view is that this variation reflects different constitutive states. However, we might imagine an alternative hypothesis: this variability could reflect different potentials of inducibility instead of different constitutive expression levels. In this line of reasoning, all reactive stocks would have a low constitutive expression of the biochemical function underlying reactivity; the inducing agents, such as those described in this paper, would reveal the more or less strong inducibility potential of the strain. The transposition of *I* factor, in a dysgenic cross, would act like a mutagenic agent; this is supported by work on bacteria showing that a single transposition event of *Tn10* triggers a detectable induction of the SOS response (ROBERTS and KLECKNER 1988). However, this interpretation disagrees with the results of LACHAUME and PINON (1993). They showed that the expression of a *I-lacZ* construct is correlated with the reactivity level of the transgenic strain, in absence of any dysgenic cross and of any inducing treatment. Therefore we may conclude that the variability of reactivity levels actually corresponds to different constitutive states. This is an important difference with the SOS response in bacteria. In normal *E. coli* strains, in absence of DNA damaging agents, the constitutive level of expression of the SOS network is always very low.

It will be of interest to investigate the effect of various classes of mutagenic agents on reactivity level. Until now, among chemical mutagens, only ethylmethanesul-

fonate has been tested, it exhibits also an enhancing effect (J.-C. BREGLIANO, unpublished data). It will be also important to know whether other kinds of stresses are able to enhance reactivity. Is this response specific for DNA damaging agents or is it a more general stress-response? Preliminary data show that reactivity might be enhanced by heat-shock too (J.-C. BREGLIANO, unpublished data). If this appears to be confirmed, it would be a further analogy with some components of the SOS network, namely the *dnaK*, *groES* and *groEL* genes (WALKER 1984).

Finally, the problem of the molecular mechanism responsible for the maternal transmission of reactivity levels is still puzzling. Two main types of molecular basis may be imagined: either true extrachromosomal determinants whose replication is more or less controlled by the nuclear genotype, or some kind of epigenetic inheritance due to DNA or chromatin modifications. For the first hypothesis we may refer to extrachromosomal circular DNA whose existence has been demonstrated in many animal species, including *Drosophila* (RENAULT *et al.* 1992). For the second hypothesis, we may imagine some kind of imprinting mechanism such as those described in other species (HOLLIDAY 1987; ALLEN *et al.* 1990). To address this question, molecular approaches are underway.

We especially thank ROLAND ROSSET for stimulating discussions and for critical reading of the manuscript. We are grateful to the Laboratoire de Physique Corpusculaire in Clermont-Ferrand and to the Centre d'Immunologie de Marseille-Luminy for kind access to gamma rays sources. A special thank to JEAN FAIN and DIDIER MIALIER for very helpful advices and to DOMINIQUE SAUVAGE and the Centre Européen de Recherches Nucléaires in Geneva for help in accurate calibration of the ^{60}Co source. We also thank ALAIN HENAUT and ANDRE BOURDILLON for help in statistical analysis of data; YANNICK AZOU and MARIE-CATHERINE TIVERON for very useful comments on the first draft and FRANCOISE GAY for excellent technical assistance. This work was supported by the Centre National de la Recherche Scientifique and the Université de la Méditerranée.

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