Metabolism and Chromosome-Break Rejoining

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Since Muller's (*1*) original discovery in 1927 that x-rays can produce gene mutations, much study has been devoted to the effects of radiation on genetic systems. One area in particular, the production of chromosomal aberrations, has been rather well quantitated. This quantitative approach began with the work of Sax (*2, 3*) and in the main was further pursued in the United States by Sax and his students and in England by Lea and Catcheside.

The results obtained by these early investigators were interpreted on the basis of a simple direct-action hypothesis. Reviews by Catcheside (*4*), Lea (*5*), and Giles (*6*) describe the development of this theory, which maintains that the ionizing radiations first break the chromosome threads. The broken ends can then (i) heal in the open position, (ii) rejoin by restitution, or (iii) rejoin with the broken ends of other chromosomes. The first of these three events leads to simple one-hit aberrations. The second is undetectable, and the third gives rise to two-hit aberrations.

It may be noted that the calculations of Lea indicate that 95 percent of the breaks originally produced restitute. Sax (*3*) observed that if a dose of radiation breaks two chromosomes in such a way that the two breaks are open simultaneously and are in close proximity then, by the process of rejoining, it is possible for a dicentric chromosome to be formed. Similarly, if the two breaks occur in the same chromosome a ring can be formed (Fig. 1). If, however, the total dosage is divided into two fractions, only part of the breaks are produced at a given time. When a long enough period elapses before the second fraction of the dose is given, this first group of breaks will restitute before the second one is produced. Therefore, the two groups of breaks will not be able to rejoin with one another to produce two-hit aberrations (Fig. 2).

In the seeds of *Vicia faba* the breaks usually stay open for the relatively long period of 30 min when they are irradiated in a vacuum and then restitute rapidly. The "open" period can be determined by the length of the period between two dose fractions that does not result in a decrease in the number of two-hit aberrations from what it would have been had the radiation been administered in one large dose. The quantitative aspects of this matter have been treated by Wolff and Atwood (*7*). Briefly, however, if the breaks from the first dose have rejoined before the second dose is administered, the yield of two-hit aberrations will be the sum of those produced by each of the two dose fractions. If the breaks stay open, the yield will be the square of the sum of the square roots of the numbers produced by each fraction.

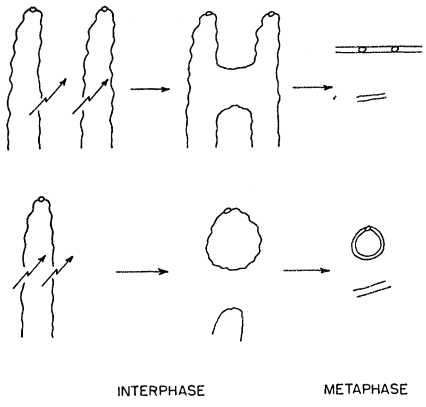

Fig. 1. Origin of two-hit aberrations. (Top) Dicentric; (Bottom) ring.

In the present experiments (*8*) we have tried to determine the mechanism of the rejoining process of broken chromosomes. Essentially, the procedure consisted of first irradiating *Vicia* seeds in a vacuum in order to produce breaks that would stay open for 30 min (*7*). Immediately after this first irradiation, various enzyme inhibitors were applied to test their effect on rejoining. Seventy-five minutes later a second fraction of radiation was administered to determine whether or not the first group of breaks was still open and capable of rejoining with the second group of breaks. Three days later, when the first mitotic root tip divisions were occurring, the roots were excised and placed in a saturated solution of paradichlorobenzene for 2 hr to accumulate metaphases. They were then fixed in C. E. Ford's modification of Flemming's fixative and stained with Feulgen reagent. Details of the technique have been given elsewhere (*7*). All irradiations were administered with a G.E. Maxitron tube operated at 250 kvp with 3 mm of Al filtration. The dose rate was 200 r/min. In each experiment 300 metaphase plates were scored for rings and dicentrics. The results are presented in Tables 1 and 2.

As may be seen in experiment 1, the breaks formed by irradiating with 600 r in a vacuum rejoin within 75 min if the material is left at room temperature (about 25°C). Actually, as noted, these breaks stay open for only 30 min. However, if the seeds are plunged into 0°C water immediately after the irradiation and kept at this temperature for the entire 75 min, ostensibly to stop all enzyme activity, the breaks stay open and are able to rejoin quantitatively with the breaks that are induced later (experiment 2). Similarly, postirradiation treatment with $2 \times 10^{-3}M$ KCN or with 95 percent CO-5 percent O_2 in the dark is able to prevent normal rejoining of the breaks, even at room temperature (experiments 3, 4, 5). If, however, the seeds in the CO mixtures are exposed to a light from a sodium arc lamp at a distance of 6 cm (720 ft-ca as measured by a Weston Illumination Meter 756 Viscor Filter), the CO inhibition of rejoining does not occur (experiments 6, 7).

These observatons indicate that the repair of radiation-induced chromosome breaks, as evidenced by rejoining, is dependent on oxidative metabolism, especially the part of oxidative metabolism that is mediated by cytochrome oxidase, which is inhibited by both KCN and CO. Consequently, the same results would occur if respiration were blocked by any mechanism other than the direct inhibition of cytochrome oxidase. In experiments 13 and 14, in which irradiated seeds were treated with a combination of $2 \times 10^{-3}M$ $Na_2S_2O_4$ and 4 to 5 mm of vacuum, the breaks did indeed stay open. Here the sodium hydrosulfite was used with the vacuum to remove completely the oxygen that might be present in the cells. Under these conditions of complete anaerobiosis, rejoining did not occur, a result that corroborates the hy-

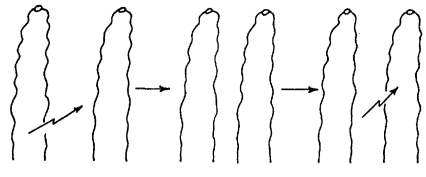

Fig. 2. Effect of dosage fractionation.

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Table 1. Effect of respiratory inhibitors on chromosome rejoining.

Expt.	Treatment immediately after dose 1 of 600 r in a vacuum at time t	Dose 2 at $t + 75$ min (r)	No. of two-hit aberrations per cell			Occurrence of rejoining within 75 min
			Observed	Expected value*		
				With rejoining	Without rejoining	
1	Room temp.	400 (air)	0.220	0.193	0.380	+
2	0°C	400 (air)	0.367	0.193	0.380	−
3	$2 \times 10^{-3}M$ KCN	400 (air)	0.367	0.236	0.440	−
4	95% CO + 5% O_2 dark	400 (air)	0.347	0.193	0.380	−
5	95% CO + 5% O_2 dark	400 (air)	0.400	0.193	0.380	−
6	95% CO + 5% O_2 light	400 (air)	0.210	0.193	0.380	+
7	95% CO + 5% O_2 light	400 (air)	0.185	0.193	0.380	+
8	Vacuum 4 to 5 mm	600 (vac.)	0.200	0.184	0.365	+
9	Vacuum 4 to 5 mm	600 (vac.)	0.143	0.184	0.365	+
10	N_2 (pyrogallol)	400 (air)	0.147	0.193	0.380	+
11	N_2 (pyrogallol)	400 (air)	0.167	0.193	0.380	+
12	2×10^{-3} BAL	400 (air)	0.123	0.157	0.293	+
13	$Na_2S_2O_4$ + vacuum	600 (vac.)	0.400	0.184	0.365	−
14	$Na_2S_2O_4$ + vacuum	600 (vac.)	0.407	0.184	0.365	−

* Values column computed from numbers of aberrations produced by dose 1 and 75 min of postirradiation treatment as expressed in the second column; also from the aberrations produced by 75 min pretreatment and dose 2. Expected values are different, since some of the pretreatments change the numbers of aberrations produced by a given dose of radiation.

Table 2. Effect of 2,4-dinitrophenol on chromosome rejoining. DNP administered immediately after dose 1 of 600 r in a vacuum (75 min before dose 2 of 400 r in air). Expected two-hit aberrations per cell with rejoining, 0.193; without rejoining, 0.380.

Molar concn. of DNP	No. of two-hit aberrations per cell
1.5×10^{-5}	0.240
1.5×10^{-4}	0.377
3.0×10^{-4}	0.367
1.5×10^{-3}	Lethal

pothesis that oxidative metabolism is necessary for rejoining. However, it must be noted that $Na_2S_2O_4$ may also inhibit respiration by reducing hydrogen transport coenzymes in the system. The end effect, however, would be the same as if its only function were to remove oxygen. In experiments 8–10 it may be observed that 4 to 5 mm vacuum or nitrogen passed over alkaline pyrogallol, as utilized, are not in themselves sufficient to deplete the intracellular oxygen content of the roots within the time limits imposed by the nature of the experiment. Under these conditions the broken ends rejoined. Similarly, treatment with $2 \times 10^{-3}M$ BAL, which removes intracellular oxygen less efficiently than $Na_2S_2O_4$ (*9*), does not prevent rejoining (experiment 12).

Table 2 demonstrates the effect of various concentrations of 2,4-dinitrophenol on rejoining. Concentrations of either $1.5 \times 10^{-4}M$ or $3.0 \times 10^{-4}M$ caused the broken ends to stay open for the duration of the experiment (75 min). At the lower concentration of $1.5 \times 10^{-5}M$, an "either-or" reaction took place, with some of the root tips exhibiting chromosome breaks that stayed open and others exhibiting breaks that closed. Consequently, we think that at the lower concentration only some roots were affected by the DNP.

In view of these results it appears that the chemical bonds formed in the process of rejoining are probably strong bonds. We believe that the probable function of oxidative metabolism in this system is to provide the high-energy phosphate bonds of ATP that are utilized to provide the energy required in the synthesis of these bonds.

Previous work by King *et al.* (*10*) has shown that CO can increase the numbers of aberrations induced in *Tradescantia* microspores. They suggest that this might be caused by the formation of hydrogen peroxide through the pathway of the flavoproteins. That carbon monoxide does not work by this pathway in the present study is shown by experiments 13 and 14, in which complete removal of oxygen by $Na_2S_2O_4$ plus vacuum, which would preclude peroxide formation, prevents rejoining.

Haas *et al.* (*11*) have suggested that cytochrome oxidase and other enzymes connected with oxidative metabolism affect the breakage of chromosomes in *Drosophila*. This suggestion resulted from experiments in which treatment with CO began before irradiation and extended for 20 min after. They also noted an intensity effect of the radiation. In the light of the present study, the results they describe might well be ascribed to a postirradiation effect of the monoxide on the rejoining of the breaks.

In summary, we would say that the rejoining of radiation-induced chromosome breaks, with its formation of chemical bonds, is inhibited by low temperature, cyanide, carbon monoxide, anaerobiosis, and dinitrophenol. We are thus led to believe that oxidative metabolism with its concurrent formation of ATP is necessary for rejoining.

References and Notes

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Generally, when some new instrument in the equipment of civilization excites wonder, it is the mystery of the instrument itself, rather than the broad sweep of the basic principles of science, upon which amazement dwells: it is with the electrical gadgeteer, for example, more often than with Maxwell or Hertz, that the marvel of radio-communication is commonly associated.—NORMAN FEATHER, *Lord Rutherford* (1940).