EXPERIMENTAL GENETICS

STRUCTURE OF CHROMOSOMES AS REVEALED BY LABELING AND IRRADIATION

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The study of the structure of the chromosome and its reproduction in the course of mitosis or meiosis is essential for the discovery of the way of interaction between mutagens and the chromosome and the mechanisms of formation of mutations, for the detection of mutations, and for the solution of other important problems. By the use of labeled thymidine, in conjunction with ionizing radiation, the number and types of chromosome aberrations arising in cells irradiated in the phases G_1, S, and G_2 of the mitotic cycle can be determined [4, 6, 8].

The object of the present investigation was to study chromosome aberrations arising in cells following irradiation in various phases of the mitotic cycle.

EXPERIMENTAL METHOD AND RESULTS

The technique was described in detail in the author's previous papers [2, 3]. Experiments were carried out to study the appearance of chromosome aberrations in cells irradiated with x-rays (dose 35 R, exposure 2 min, apparatus RUP-1, filters Cu 0.5 mm and Al 0.5 mm) in the phases G_1, S, and G_2 of the mitotic cycle. The test object was the cells of a culture of mouse embryos of line C_3Hf Pu II. The phase of the mitotic cycle at the moment of irradiation was determined from the incorporation of thymidine-H^3, introduced into the culture for 15 min. Irradiation was carried out immediately after addition of thymidine-H^3. Some experiments were carried out without the use of the label.

The curve showing the changes in the number of labeled mitoses in the irradiated culture are shown in Fig. 1. To analyze it, the duration of the mitotic cycle of the cell (mean value 23±2 h) and of its phases ($M + G_2$ = 5.5 h; S = 10.5 h; G_1 = 7-8 h) was calculated.

Elsewhere [3] the author discussed the possible changes in the duration of the mitotic cycle of cells, the possible variations in the mean duration of the cycles from experiment to experiment, and the effect of irradiation (35 R) on the cycle. The analysis of the chromosome aberrations undertaken in the present study took all these phenomena into account. The dose of radiation used hardly affected the duration of the cycle of the irradiated cells at all.

The incidence and the types of the chromosome aberrations were studied (Tables 1 and 2). Particular attention was paid to the aberrations giving evidence of the structure of the chromosomes: chromatid (single) bridges, revealing the double structure of the chromosome, and the chromosome (double) bridges. The latter are considered to be structures arising at the time when the chromosome reacts to irradiation as a single strand.

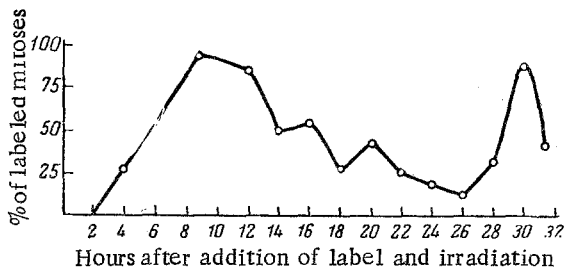

Fig. 1. Changes in the number of labeled mitoses in a culture at various times after irradiation (label introduced for 15 min before irradiation).

The largest number of cells with aberrations was observed 4 h after irradiation, and rather fewer were found 2 h after. At this time cells irradiated in the phase G_2 were beginning to undergo mitosis. The percentage of chromatid bridges was very high in this case, but the appearance of chromosome bridges was completely unexpected. The mean percentage of chromosome bridges 2-4 h after irradiation was 0.602±0.16 (0.133±0.01 in the control). The difference between these values (0.469) was 2.39 times greater than the error (0.16). This evidently demonstrates that sometimes

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TABLE 1. Chromosome Aberrations Arising in the Anaphase of Cells after Irradiation

Time after irradiation (in h)	Phases in which most cells analyzed were irradiated	Number of anaphases analyzed	Percent of cells with aberrations	Percent of chromosome bridges	Percent of chromatid bridges	Percent of single fragments	Percent of paired fragments
Control		7 644	4±0,24	0,133±0,01	1,07±0,13	0,76±0,1	2,11±0,17
2	G_2	1 042	12,6±1,02	0,576±0,19	5,37±0,7	2,3±0,46	5,6±0,71
4	G_2	1 275	21±1,14	0,627±0,21	8,2±0,77	3,2±0,49	4,4±0,57
6	G_2—S	1 330	8,1±0,75	0,15±0,11	2,7±0,44	1,1±0,29	3,2±0,48
9	S	1 306	10±0,82	0,15±0,11	3,75±0,53	0,54±0,21	2,1±0,4
12	S	1 001	10,8±0,97	0,5±0,22	3,4±0,58	0,5±0,22	7,1±0,81
16	S—G_1	1 277	11,7±0,9	0,942±0,27	3,37±0,51	1,1±0,29	4,9±0,61
18	S—G_1	1 148	11,5±0,95	1,4±0,35	3,57±0,55	1,3±0,34	4,5±0,61
20	G_1	536	10 ±1,3	1,2±0,45	2,05±0,62	0,56±0,32	5,4±0,98
22	G_1	1 395	8,74±0,76	0,935±0,26	2,8±0,44	1,15±0,39	3,3±0,48
24	G_2	1 519	7,63±0,68	1,05±0,27	2,04±0,36	0,73±0,22	4,1±0,51
26	G_2	1 010	4,07±0,62	1,39±0,37	0,994±0,32	0,3±0,17	1,6±0,4
28	G_2—S	3 031	7,7±0,48	1,5±0,21	1,5±0,22	0,6±0,14	4,36±0,37
30	S	1 261	7,0±0,72	0,784±0,25	2,15±0,41	0,4±0,2	4,4±0,58
45		1 876	4,0±0,31	0,406±0,14	0,426±0,15	0,64±0,18	2,77±0,37
50		795	3,53±0,66	0,252±0,17	0,755±0,31	0,5±0,25	2,52±0,56
58		1 050	7,9±0,83	0,572±0,23	1,71±0,4	1,62±0,39	5,6±0,71
70		520	3,72±0,83	0,385±0,27	1,54±0,53	1,53±0,54	1,3±0,5

TABLE 2. Number of Chromosome Aberrations in Labeled (L) and Unlabeled (UL) Cells of an Irradiated Culture of Mouse Fibroblasts

Time after irradiation (in h)	Cells	Phase in which irradiated	Percent of chromatid bridges	Percent of chromosome bridges
16—22	L	S	3,14±0,67	0,6±0,3
	UL	G_1	1,83±0,36	1,22±0,3
28—31	L	S, X_2	3,48±0,63	0,36±0,2
	UL	G_2, X_2	0,57∓0,2	0,64±0,21
Control	—	—	1.07±0,13	0,13±0,014

the chromosome may react to irradiation as a double and a single structure simultaneously. A fall in the number of cells with aberrations and a decrease in the percentage of chromatid bridges were observed 6 h after irradiation. The percentage of chromatid bridges increased 9 h after irradiation and remained at the same level until 18 h, falling again after 20 h to a new level still significantly different from the control value. After 18-24 h most of the dividing cells were those irradiated in phase G_1. It is clear from Table 2 that chromatid bridges were formed in the cells irradiated in the phases S and G_1; they were found in labeled and unlabeled anaphases.

Analysis of the incidence of chromosome bridges showed that their massive appearance in the anaphases was connected with the beginning of mitoses among the cells irradiated in the phase G_1 (see Tables 1 and 2). It will be noted that the second peak in the number of cells with aberrations did not coincide with the peak of the number of labeled cells, and, consequently, it was not entirely due to the cells irradiated in the S phase, but evidently also to cells irradiated in the late G_1 phase. The percentage of chromatid bridges at the different times of fixation showed close correlation with the percentage of cells with aberrations. This shows that the greater part of the aberrations were formed as chromatid aberrations.

The mitotic cycles of the cells in the culture investigated lasted on the average 23±2 h, so that it may be concluded that from 24 h (see Fig. 1) after introduction of the label and irradiation, mainly second mitoses of the

cells X_2 were present. Evidently 24, 26, and 28 h after the beginning of the experiment, mainly mitoses of the cells X_2 irradiated in the G_2 phase were observed. This conclusion was confirmed by the new rise of the curve of labeled mitoses at the 28th-30th h, and also by the fact that the reduction in the number of granules of label per cell by 50% (after introduction of the label) took place in the present experiments after 22 h. Cytological analysis of the aberrations after 24 h (see Table 1) and analysis of the aberrations in the labeled and unlabeled anaphases in the preparations from the autoradiographs (see Table 2) showed that no chromatid aberrations appeared in X_2 in the cells irradiated in the G_2 phase. It is clear from Tables 1 and 2 that a high percentage of chromatid bridges appeared in X_2 in the cells irradiated in the S phase. The appearance of chromosome bridges and paired fragments in the X_2 cells irradiated in G_2 is nothing unusual, for they may appear in X_2 as "surviving" aberrations from X_1. In fact, an increase in their number was observed by comparison with the control level in later periods: after 30, 45, 50, and 58 h.

In contrast to these aberrations, the chromatid bridges could not "survive" from mitosis to mitosis. Their appearance in the anaphases must be associated with the fact that aberration may arise in cells not only in X_1, but also in X_2. This property was possessed by the cells irradiated in the S phase. Possibly the fact that the number of cells with aberrations fixed after 58 h was greater than in the control may also be due to cells irradiated in the S phase. The appearance of chromosome aberrations in a series of successive mitoses after a single exposure to radiation has been described by the author as "sustained mutability."

In 1964, N. P. Dubinin and E. G. Saprykina [1] put forward the hypothesis that after treatment with a chemical mutagen, chromosome aberrations may arise de novo in a series of successive mitoses of the cells.

The author considers that the formation of chromatid aberrations in X_2 cells irradiated in the S phase is associated with the fact that during synthesis the chromosome is very sensitive to the action of extraneous agents. During irradiation, free radicals, peroxides, antimetabolites, and so on appear in the cells. These extraneous agents may injure the newly synthesized complementary half of the chromatid (Fig. 2, in which the newly synthesized half of the chromatid is shown by a broken line). Since such injuries occur at the subchromatid level, their effect will be seen more clearly at the chromatid level only after subsequent replication of the chromosome. Another possible mechanism of the effects of primary intrachromosomal injuries has been discussed by the author elsewhere [2, 3].

In recent years reports have been published of the appearance of chromosome aberrations in cells irradiated in phases G_1, S, and G_2 of the cycle [4, 6, 8]; the present findings in relation to the appearance of chromatid and chromosome bridges in irradiated cells are in agreement with them. Splitting of the chromosome takes place at the end of phase G_1. Evidently for DNA synthesis to begin, the chromosome must split. In accordance with this, the structure of the chromosome may be represented schematically as in Fig. 2.

This model of the structure of the chromosome is also convenient for explaining the effect of chemical mutagens with delayed action (alkylating compounds) on the chromosome. In this case, for the abberation to be produced, the chromosome must have passed through the S phase [5, 7], and only chromatid abberations can arise.

In the author's opinion, the difference between the action of radiation and of chemical mutagens of delayed action lies in the "degree of injury" to the chromosome arising following the action of the mutagenic factor on it. The ionizing particle, as it flies past, may induce structural disturbances in both sub-strands of the chromosome in the G_1 phase (the chromosome will react to irradiation as a single strand). The molecule of the chemical mutagen induces a disturbance in only one sub-strand of the chromosome. Such a disturbance may spread to the next sub-strand after reduplication in the S phase and, as a result of the semiconservative mechanism of reduplication of the chromosomes, it may affect the whole chromatid in X_1. A disturbance arising in the G_2 phase can be detected only in X_2.

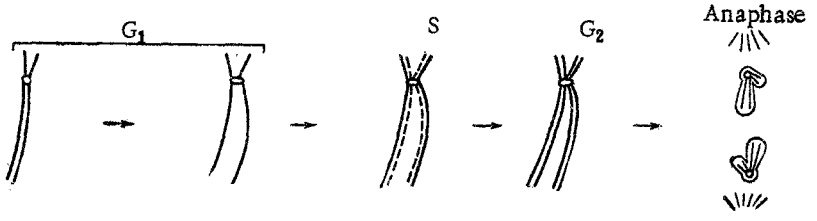

Fig. 2. Structure of the chromosome at different phases of the mitotic cycle.

With this mechanism of formation of aberrations, chromosome aberrations cannot in general appear (if they are formed in the G_1 phase) or they must appear extremely rarely, only if two molecules of the mutagen should happen to fall into the identical loci of the two strands of the chromosome in the G_1 phase (if the final formation of these aberrations takes place in the later stages of the cycle, in S and G_2).

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