RADIATION-INDUCED CHROMOSOME BREAKAGE, DESOXYRIBOSE-NUCLEIC ACID SYNTHESIS AND THE MITOTIC CYCLE IN ROOT-MERISTEM CELLS OF *VICIA FABA*

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(With 2 figures in the text)

1. Introduction

Variation of the reactions of chromosomes to ionizing radiations with variation of the time interval between irradiation and fixation has received some attention, because of its importance to theories concerning the action of the radiations, and because it seems likely to help us to understand the physiological and morphological changes occurring during the mitotic cycle. Interpretation of the relevant data, however, is not easy, for it is difficult to determine the precise relationship between the timing of the mitotic cycle and the timing of the radiation treatment.

Howard & Pelc (1952) have now published some results of studies of the incorporation of ^{32}P labelled desoxyribose-nucleic acid in the chromosomes of irradiated *Vicia faba* root tips. These roots were of the same variety and were cultured under the same conditions as those which the present author has used to study chromosome breakage. Valid comparisons may therefore be made, and these have proved of considerable interest. They seem to confirm that Howard & Pelc have discovered a phase of the mitotic cycle that is of great significance.

2. The synthesis period

Fig. 1*a* summarizes the conclusions of Howard & Pelc. They found that the mitotic cycle could be divided into four phases. In untreated roots mitosis proper occupies 4 hr. In the figure this is represented by two 2 hr. periods, D_1, the post-metaphase period of one division, and D_2, the prophase period of the succeeding division. After division there is a period of 12 hr., G_1, that precedes a period S, lasting 6 hr., during which latter synthesis of DNA occurs. S is followed by another period, G_2, of 8 hr. before the onset of prophase.

Irradiation with 145 r. X-rays altered the mean duration of these periods and had the effect of dividing the cells into two populations, one of which is delayed in G_1 and the other delayed in G_2 (*A* and *B* in Fig. 1*a*). These results lead to the conclusion that most irradiated cells are in the synthesis period sometime between 10 and 20 hr. before metaphase. This period is hatched in the remainder of Fig. 1 and is referred to below as the synthesis period.

3. EXPERIMENTAL CONDITIONS OF THE CHROMOSOME-BREAKAGE STUDY

These have already been described and some of the results published in Thoday (1951, 1952). Briefly, beans were irradiated at three dose-levels (52·5, 104·6 and 210 r. each in 4 min.) with X-rays, and at three with α-particles from Radon (8·5, 15·3 and 27·9 energy units). The irradiations were carried out by Dr L. H. Gray. Tips were then fixed 1½, 3, 6, 9, 12, 18, 24, 36 and 48 hr. after each treatment, and chromosome aberrations were classified at metaphase into the types described in Thoday (1951). The more

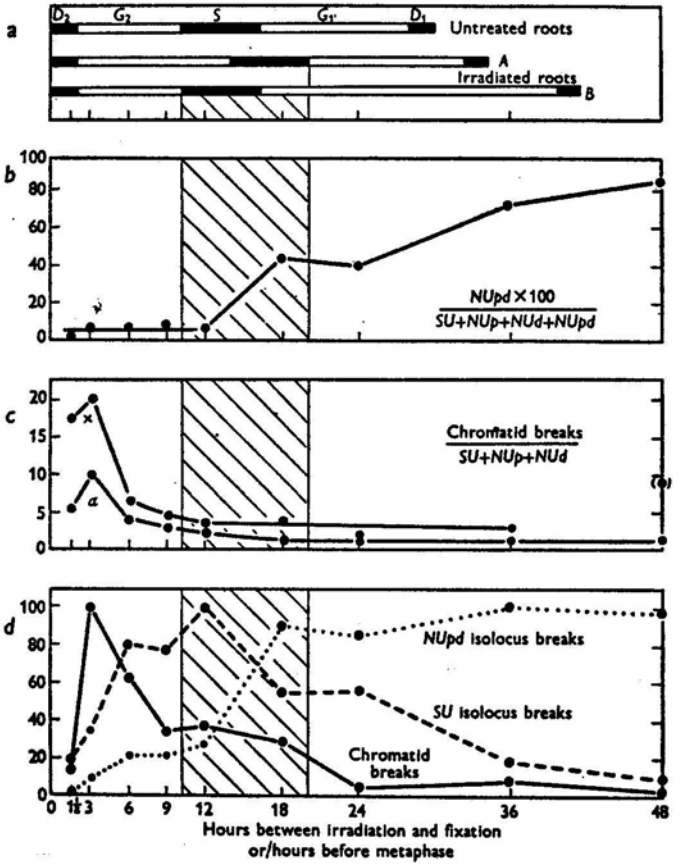

Fig. 1. For descriptions see text. Note: in *b* to *d* the abscissae represent the time elapsing between irradiation and fixation of the roots. Cells irradiated at later times were at *earlier* stages of mitosis; so that mitosis must be regarded as proceeding from right to left and as reaching metaphase at the extreme left. *a* is drawn to correspond: cells proceed from division D_1 at the right to Division D_2 at the left.

important types are illustrated in Fig. 2. The irradiations are not the same as those used in Howard & Pelc's experiments, but as the X-ray doses span those used by Howard & Pelc, useful comparisons can be made.

4. ISOLOCUS BREAKS

Table 1 lists, and Fig. 1*b* illustrates, the proportion of isolocus breaks that are *NUpd* in type, that is to say, show no evidence of sister-chromatid union. This proportion varies with the time-interval between irradiation and fixation. Converting the proportions

by sine transformation permits computation of the analysis of variance set out in Table 2. There is no significant variation due to irradiation treatment (dose-level or α-particle X-ray comparison).* There is highly significant variation due to time-interval between irradiation and fixation, the greater part of which is due to difference between material fixed 12 hr. or less after irradiation and that fixed 18 hr. or more after irradiation. There

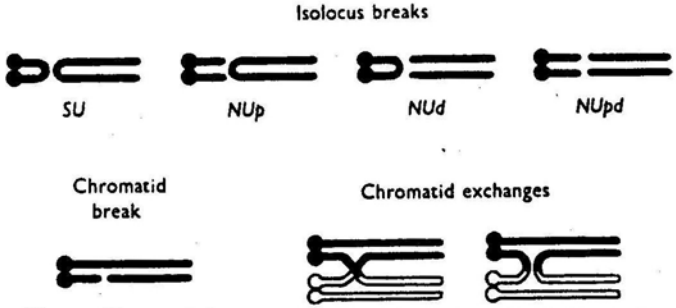

Fig. 2. Types of chromosome-aberration referred to in the text.

Table 1. *The proportion of isolocus breaks that are NUpd*

Time (hr.)		1½	3	6	9	12	18	24	36	48
X-rays										
52·5 r.	Total isolocus breaks	2	1	5	11	15	12	14	8	6
	% *NUpd*	0	0	0	0	27	67	64	62	100
104·6 r.	Total isolocus	6	8	31	16	12	24	18	29	7
	% *NUpd*	17	12	3	12	17	46	44	79	86
210 r.	Total isolocus	0	16	37	32	38	38	26	16	3
	% *NUpd*	0	12	8	9	3	26	77	56	100
α-particles										
8·5 e.u.	Total	5	7	11	17	16	24	44	19	15
	% *NUpd*	0	14	18	29	0	54	41	84	80
15·3 e.u.	Total	9	11	34	22	36	44	29	11	17
	% *NUpd*	11	18	24	27	22	55	10	100	82
27·9 e.u.	Total	3	9	34	45	33	19	31	30	16
	% *NUpd*	0	0	12	11	9	57	39	73	94

Table 2. *Analysis of the variance of the percentage of isolocus breaks that are NUpd*

Source	Sum of squares	*N*	Mean square	*F*	*P*
1½–12 hr. against 18–48 hr.	18236·1370	1	18236·14	108·86	Very small
Residual due times	4477·5508	6	746·26	4·47	$<0.01>0.001$
Total due times	22713·6878	7	3244·81	19·37	Very small
Treatments	205·5035	5	41·10	—	—
Error	5862·7537	35	167·51	—	—
Total	28781·9448	47[1]			

[1] The data for 1½ and 3 hr. were combined for this computation.

is significant residual variation due to times, but this is all due to differences within the material fixed 18 hr. and more after irradiation. The data for 12 hr. and less are homogeneous.

The probability that an isolocus break shall be *NUpd* is therefore high in material irradiated 48 hr. before fixation, decreases somewhat as the interval is reduced to 18 hr.,

* Cf. Kotval & Gray (1947), who reach different conclusions using *Tradescantia*.

then decreases suddenly, remaining approximately constant thereafter. It is striking that the sudden decrease occurs in the synthesis period.

Strong evidence has been published elsewhere (Thoday, 1952) that in this material most *NUpd* breaks are chromosome breaks (that is have been produced from chromosomes irradiated before they have split into chromatids) and that most isolocus breaks showing evidence of sister-union are isochromatid breaks (that is have been produced from chromosomes irradiated after they have split). It follows that the reproduction of the chromosomes occurs at one end of or during the synthesis period. The lack of significant variation of the proportion of isolocus breaks in the material fixed 12 hr. and less after irradiation indicates that by the time the synthesis period is complete chromosome reproduction has occurred in most cells.

Table 3. *Chromatid break: isochromatid break ratios at different fixation times*

Time (hr.)	1½	3	6	9	12	18	24	36	48
α-rays:									
Chromatid breaks	91	252	276	225	165	65	72	19	9
Isochromatid breaks	16	24	65	68	74	39	45	11	7
Ratio	5·7	10·05	4·3	3·3	2·2	1·7	1·6	1·7	1·3
X-rays									
Chromatid breaks	123	437	450	237	205	183	34	49	9
Isochromatid breaks	7	22	69	54	58	45	21	16	1
Ratio	17·6	19·9	6·5	4·2	3·5	2·5	1·6	3·1	9

5. CHROMATID : ISOCHROMATID BREAK RATIOS

Table 3 lists, and Fig. 1*c* illustrates, the ratios of chromatid breaks to isochromatid breaks (*SU*+*NUp*+*NUd* isolocus breaks) found in the material fixed at the different intervals after irradiation. These ratios are higher for X-ray-treated than for alpha-particle-treated material as is to be expected on the basis of the target theory. They do not appear to vary when the time between irradiation and fixation is varied between 12 and 48 hr., but they increase strikingly as the time interval is reduced below 12 hr., reaching a peak value at 3 hr.

This evidence indicates that events of importance are occurring during phase G_2 of Howard & Pelc. It appears that some new process follows the completion of synthesis and continues until prophase begins. Howard & Pelc (1952) have already argued that important physiological events occur in this phase.

This change in the ratios seems best interpreted with Sax & Swanson (1941). They explained similar observations by supposing that there is a gradual separation of the chromatids which reduces the probability that a particular ionizing particle passing through the target volume of one chromatid shall pass through that of the other chromatid. Whether the drop in ratio at 1½ hr. is significant is not certain. If so it might indicate that there is some conjugation of the chromatids at the onset of mitosis similar to that which occurs between chromosomes in meiotic prophase.

6. SENSITIVITY OF THE CHROMOSOMES

Fig. 1*d* represents the variation of the yields of different kinds of aberration with variation in the time interval between X-irradiation and fixation. The curves are constructed as follows. The yield per 100 cells of a particular aberration at any particular

fixation time is represented as a percentage of the yield for the time at which the yield is maximal.

It will be seen that the yield of *NUpd* isolocus breaks is maximal to the right of the synthesis period, that is in cells irradiated before they have reached the synthesis period. The yield of *SU* isolocus breaks is maximal in cells irradiated when they are near the end of the synthesis period. The yield of chromatid breaks is maximal in cells irradiated long after the synthesis period and chromosome reproduction are complete. The curve for chromatid exchanges is not included since it is essentially the same as that for chromatid breaks.

A small proportion of the chromatid breaks and exchanges and a rather larger proportion of the *SU* isolocus breaks occurs in cells that would appear to have been irradiated before they have reached the synthesis period. Some of the cells concerned may in fact have been irradiated after the synthesis period. Irradiation in prophase is known sometimes to reverse mitosis (Carlson, 1940) and also there may be a small proportion of cells that spend much longer than the average in interphase. Either of these possibilities might result in chromatid aberrations in the material fixed more than 24 hr. after treatment. It does not seem, however, that such explanations can account for all the observed *SU* aberrations in material fixed 24 hr. or more after treatment, and it seems likely that some of these aberrations are produced by a mechanism involving interference with chromosome reproduction, as suggested by Darlington & La Cour (1945). It is, however, quite clear that most such aberrations are produced by a mechanism that does not involve the process of chromosome reduplication.

We here underline what may prove to be a fundamental difference between ionizing radiations and certain radiomimetic chemicals (McLeish, 1952; Revell, 1952). The chemicals appear only capable of causing aberrations if applied before the synthesis period. Irradiations can produce them after it is ended. Quite possibly irradiations when applied before synthesis may produce some aberrations by a mechanism comparable with chemical mutagenesis, but it seems clear that the mechanisms are usually different. Chemical mutagenesis may be tied up with the metabolism of synthesis, most radiation chromosome breakage is not.

7. Conclusion

Though the data presented in Fig. 1 do have an important bearing upon theories of chromosome breakage, the primary function of this paper is rather to bring out their bearing on the assessment of Howard & Pelc's conclusions concerning the time at which DNA synthesis occurs. This is necessary since different workers using different techniques and materials have published results that seem to indicate that the synthesis of chromosomal nucleic acid occurs at other stages of the mitotic cycle. In particular there seems to be a definite contradiction between the results of Lison & Pasteels (1951), who concluded that the DNA content of nuclei doubles in telophase, those of Ris (1947) who obtained evidence for doubling in prophase, and those of Howard & Pelc. It is therefore interesting to find that, whatever interpretation is put on the chromosome breakage data, the curves in Fig. 1 provide strong and independent evidence that Howard & Pelc have discovered a most important phase of the mitotic cycle.

8. SUMMARY

1. Evidence is put forward that, in *Vicia faba* root-tips chromosome reduplication takes place during the period of interphase in which, according to Howard & Pelc, chromosomal nucleic acid is synthesized.

2. During the subsequent part of interphase there is evidence that separation of the chromatids is occurring.

3. It is during the part of interphase subsequent to DNA synthesis and chromosome reduplication that most radiation-induced chromatid breaks and exchanges and *SU* isolocus breaks are initiated.

REFERENCES

CARLSON, J. G. (1940). Immediate effects of 250 r. of X-rays on the different stages of mitosis in neuroblasts of the grasshopper, *Chortophage viridifasciata*. *J. Morph.* **66**, 11.

DARLINGTON, C. D. & LA COUR, J. F. (1945). Chromosome breakage and the nucleic acid cycle. *J. Genet.* **46**, 180.

HOWARD, ALMA & PELC, S. R. (1952). Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *Heredity*, **6**, suppl., p. 261.

KOTVAL, J. P. & GRAY, L. H. (1947). Structural changes produced in microspores of *Tradescantia* by alpha-radiation. *J. Genet.* **48**, 136.

LISON, L. & PASTERLS, J. (1951). Études histophotometriques sur la teneur en acide desoxiribonucléique des noyaux au cours du développement embryonnaire chez l'Oursin *Paracentrotus lividus*. *Arch. Biol.* **62**, 1.

MCLEISH, J. (1952). The action of maleic hydrazide in *Vicia*. *Heredity*, **6**, suppl., p. 125.

REVELL, S. H. (1952). Chromosome breakage by X-rays and radiomimetic substances in *Vicia*. *Heredity*, **6**, suppl., p. 107.

RIS, H. (1947). The composition of chromosome during mitosis and meiosis. *Cold Spr. Harb. Symp. quant. Biol.* **12**, 158.

SAX, K. & SWANSON, C. P. (1941). Differential sensitivity of cells to X-rays. *Amer. J. Bot.* **28**, 52.

THODAY, J. M. (1951). Effect of ionising radiations on the Broad Bean Root. Part IX. *Brit. J. Radiol.* **24**, 572, 622.

THODAY, J. M. (1952). Sister-union isolocus breaks in irradiated *Vicia faba*: the target theory and physiological variation. *Heredity*, **6**, suppl., p. 299.