The author expresses his appreciation to Dr. James F. Crow for valuable help during the course of this work. Thanks are also due to Dr. E. R. Immel for his helpful suggestions.

* Contribution No. 84 of the National Institute of Genetics, Mishima-shi, Japan.

† Contribution No. 570. This work was supported by a grant from the University Research Committee from funds supplied by the Wisconsin Alumni Research Foundation.

[1] R. A. Fisher, *Proc. Roy. Soc. Edinburgh*, **42**, 321–341, 1922.

[2] S. Wright, *Genetics*, **16**, 97–159, 1931.

[3] R. A. Fisher, *The Genetical Theory of Natural Selection* (Oxford, 1930).

[4] M. Kimura, *Genetics*, **39**, 280–295, 1954.

[5] S. Goldberg, Ph.D. thesis, Cornell University, 1950.

[6] A. Robertson, *Genetics*, **37**, 189–207, 1952.

[7] M. Morse and H. Feshbach, *Methods of Theoretical Physics* (New York, 1953).

[8] S. Wright, these PROCEEDINGS, **31**, 382–389, 1945.

[9] W. Feller, *Proc. Second Berkeley Symposium on Math. Stat. and Prob., Univ. of California*, pp. 227–246, 1951.

---

*AN X-RAY ANALYSIS OF CHROMOSOME DUPLICATION**

BY KARL SAX AND EDWARD D. KING

ARNOLD ARBORETUM AND BUSSEY INSTITUTION, HARVARD UNIVERSITY, JAMAICA PLAIN, MASSACHUSETTS

Communicated January 5, 1955

The induction of half-chromatid aberrations by X-rays provides evidence regarding the multiple nature of the chromosome and may be of considerable significance in the analysis of chromosome duplication. The occurrence of half-chromatid exchanges was first described by Swanson[1] in 1943. These aberrations, found in pollen-tube mitoses of *Tradescantia*, were of sporadic occurrence. Recently Crouse[2] has found that practically all the X-ray-induced aberrations induced at the first meiotic metaphase stage in *Lilium* involve half-chromatid breaks and exchanges. Breaks in one of the half-chromatids of each of two chromatids are followed by reciprocal translocation to produce a chromatid bridge at anaphase. The close association of the coiled half-chromatids prevents the terminal separation of the two anaphase chromosomes (cf. Fig. 1).

Occasional half-chromatid aberrations have been found at anaphase in the division of the microspore nucleus of *Tradescantia* following X-irradiation. The dosages commonly used (50–150 r) produced considerable stickiness of the chromosomes, so that accurate analysis of chromosomal aberrations could not be made until about 6 hours after raying. By reducing the dosage to 25 r and keeping the inflorescences at 3° C. during irradiation, it was possible to obtain clear figures of mitoses and induced aberrations as early as 3 hours after raying. Irradiation at 3° C. was done to compensate for the low dosage, since it has been shown that the chromosome aberration frequency can be increased by raying at low temperatures.[3]

Chromosomal aberrations induced at 4–6 hours before anaphase consisted almost entirely of half-chromatid bridges at anaphase. Presumably, these chromosomes were irradiated at prometaphase or metaphase. At these stages the two sister

chromatids have developed into sister chromosomes, which later pass to opposite poles at anaphase. The visual evidence provides little or no indication that each of the sister chromosomes is bipartite, but the X-ray evidence proves that each consists of two half-chromatids. Breaks in a half-chromatid of each sister chromosome are followed by a reciprocal interchange or a lateral fusion between a half-chromatid of one chromosome and a half-chromatid of the other. It has not been possible to determine which type of aberration is produced, but, judging from the types of chromatid aberrations induced at prophase, both occur. In either case a bridge is formed at anaphase with no free fragment. If there were a reciprocal exchange of half-chromatids, the daughter chromosomes at anaphase would not be able to separate because of the close association of the half-chromatids in a common coil (see Fig. 1). If a dicentric half-chromatid were formed, it would produce a bridge at anaphase and the acentric half-chromatid fragment would be held in close association with the unbroken sister half-chromatids.

As shown in Table 1, the transition between half-chromatid aberrations and chromatid aberrations appearing at anaphase occurs between 6 and 8 hours after irradiation. After about 8 hours only chromatid aberrations appear at metaphase and anaphase. The chromatid aberrations are induced at prophase when the

TABLE 1

TRANSITION FROM HALF-CHROMATID TO CHROMATID ABERRATIONS IN *Tradescantia* MICROSPORES FOLLOWING X-RAY DOSE OF 25 r AT 3° C.

HOURS AFTER EXPOSURE	TOTAL CHROMOSOMES	TYPES AND FREQUENCIES OF ABERRATIONS						
		Half-Chromatid			Chromatid			
		Bridge	Exch.	Per Cent	Iso.	Exch.	Del.	Per Cent
4	888	67	6	8.2	0	0	0	0
5	366	20	0	5.6	0	0	0	0
6	408	22	0	5.4	0	0	1	0.2
7	786	33	2	4.5	9	0	0	1.0
8	1386	6	0	0.4	52	6	23	10.2
10	324	0	0	0	14	5	4	7.1
12	480	0	0	0	16	1	5	4.6

chromosomes are clearly differentiated into two sister chromatids. One or both may be broken at any given locus to produce deletions of one or both chromatids, or broken ends of chromatids in different chromosomes may unite to produce reciprocal translocations or dicentric chromatids. Only chromatid aberrations are found in microspores fixed at 10–27 hours after irradiation. At 27–30 hours some chromosome aberrations are found, and the transition from chromatid to chromosome aberrations continues for about 10 hours, varying with radiation dosage and environmental factors such as temperature and light. After the transition only chromosome aberrations are found.[4]

The "chromosome aberrations" are induced during the resting stage. The breaks and unions induced at this time appear at metaphase and anaphase as chromosome deletions in the form of double rods or rings, centric ring chromosomes, and dicentric chromosomes. These aberrations involve the chromosome as a single unit, and the X-ray evidence would indicate no bipartite structure at the resting stage. The chromosome aberrations continue for 5–8 days after raying the microspores, depending upon environmental conditions. Aberrations induced at the preceding meiosis are rarely recovered in microspore nucleus divisions because of the lethal effect of the deletions.

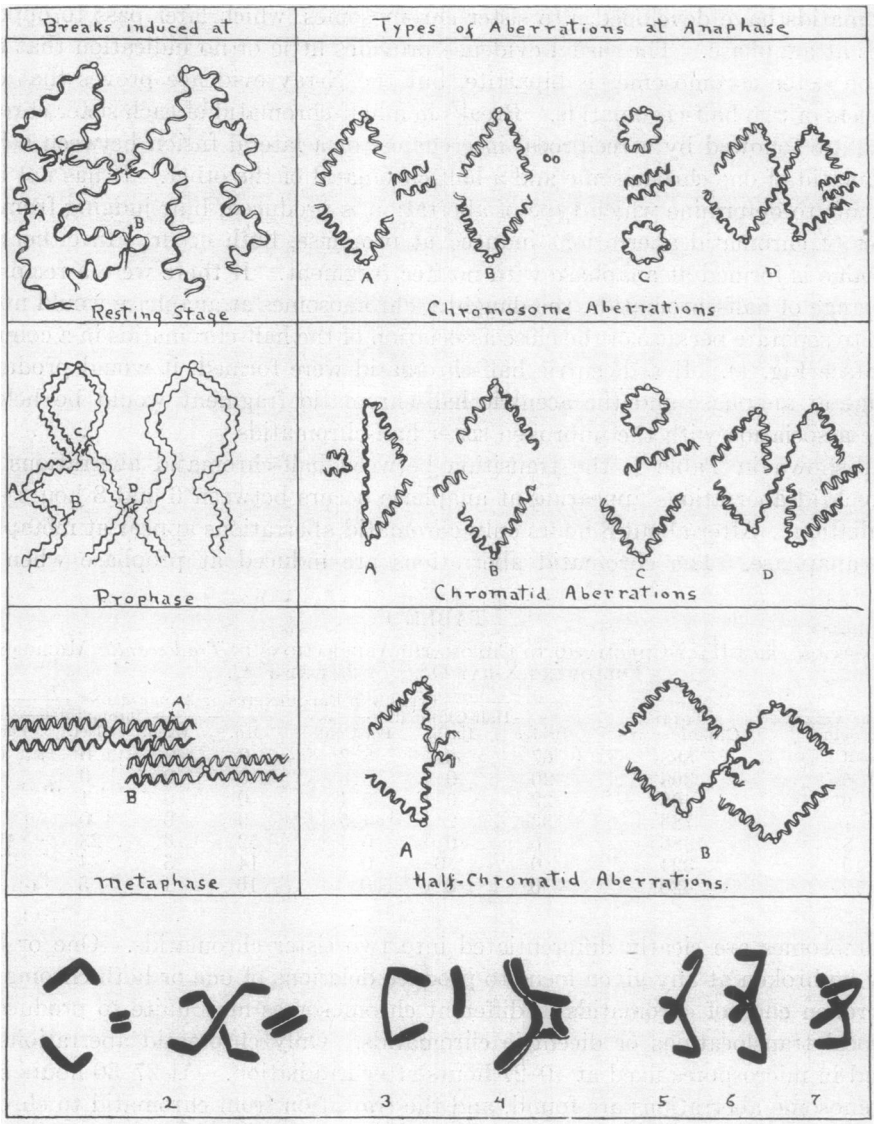

FIG. 1.—Types of chromosomal aberrations induced by X-rays at various stages of the nuclear cycle of *Tradescantia* microspores. The breaks induced at the resting stage involve both sister chromatids at any one locus and may lead to the formation of rod or ring deletions or to exchanges between chromosomes or chromosome arms to produce centric ring and dicentric chromosomes. Breaks induced at prophase may involve one or both of the sister chromatids at a given locus, which may lead to the deletion of a single chromatid or to the deletion of both chromatids. The isochromatid deletion is usually accompanied by lateral fusion to form a chromatid bridge and fused deletions at anaphase. Exchanges between chromatids of different chromosomes of chromosome arms results in chromatid rings and chromatid dicentrics, with the accompanying deletions. Aberrations induced at prometaphase involve only one of the half-chromatids of each sister chromosome and result in bridges with no free fragment at anaphase.

Camera lucida drawings of some of these types of aberrations are shown in sections *1–7* of the figure. Chromosome aberrations: *1*, deletion; *2*, dicentric. Chromatid aberrations: *3*, isochromatid; *4*, chromatid dicentric. Half-chromatid aberrations: *5*, *6*, *7*—bridges found at anaphase by exchange or lateral fusion of half-chromatids.

Thus the X-ray-induced aberrations would indicate that the chromosomes of the *Tradescantia* microspore are single threads during the resting stage, double at prophase, and four-partite at prometaphase and metaphase. But, if the chromosome is four-partite at metaphase, each of the daughter chromosomes must be two-partite, and the chromosome must go into the resting stage as a two-strand thread.

There are several lines of evidence to show that the X-ray effects are not sufficiently restricted in space to serve as a precise tool for analyzing the finer details of chromosome structure. A single "hit" can break one or both of two sister chromatids at anaphase when they are microscopically differentiated into two separate threads and are separated by at least 0.2 μ. During early prophase, when the sister chromatids are close together, both are likely to be broken at any one locus, but as prophase progresses and the sister chromatids become more separated, the frequency of isolocus breaks decreases and the frequency of aberrations involving a break in only one of the two sister chromatids increases. Lea[5] has estimated that 15–20 ionizations are needed to produce a chromosome break and that a single effective "hit" covers a diameter of 0.9–1.3 μ.

The bipartite structure of the resting-stage chromosome is indicated by indirect cytological evidence. Most cytologists are agreed that the anaphase chromosome consists of at least two chromatids which are intertwined in a common coiled structure.[6] The X-ray evidence shows the metaphase chromosome to be four-partite, and thus the anaphase chromosome must be bipartite. Yet the resting-stage chromosome responds to X-ray breakage as a single unit. It is not surprising that the resting-stage nucleus should react to X-rays as a single unit in view of the relatively large area effected by a single "hit." But why do each of the sister chromatids at metaphase react as a bipartite structure when the half-chromatids of each chromatid must be very closely associated? This association of half-chromatids at metaphase certainly is closer than that of sister chromatids at prophase, when X-rays can break one or both of the threads at the same locus. The half-chromatids at metaphase would be expected to be more closely associated than are the sister threads in the resting-stage chromosome; yet in the latter stage both threads are invariably involved in X-ray-induced aberrations.

The metaphase chromosome is four-partite, but only one of each of the sister half-chromatids is involved in the X-ray-induced aberrations. If both were broken at the same locus, we should get simple deletions; but such aberrations are very seldom found. It is possible that such aberrations do occur but that the deletion is held in place by the chromosome pellicle and does not become a free fragment until the chromosome sheath disintegrates at telophase or interphase. At metaphase each of the two sister chromosomes presumably is inclosed by its own pellicle, yet half-chromated exchanges do occur between sister chromosomes. Exchanges between both half-chromatids of the two sister chromosomes might be expected to occur if both are broken, but these types of aberrations are not found. Chromatid types of aberrations do not appear at metaphase and anaphase until 7 or 8 hours after irradiation.

The peculiar behavior of the metaphase chromosomes in response to X-irradiation may be related to the time of chromosome duplication. The evidence from the types of X-ray-induced aberrations would indicate that the duplication of the chromosome occurs shortly before metaphase. According to Swift,[7] the DNA

content of the *Tradescantia* microspore nucleus is doubled just prior to nuclear division. However, autoradiographic analyses of *Tradescantia* microspores made by Taylor[8] show that P^{32} is incorporated in the chromosomes at about 26–32 hours before metaphase. This is the time when the X-ray-induced aberrations shift from chromosome types to chromatid types. Howard and Pelc[9] have found that P^{32}-labeled DNA is incorporated in the chromosomes of *Vicia faba* root tips during this transition period, which in their material was 10–20 hours before metaphase. It is possible that chromosome synthesis begins at the onset of this transition period but that chromatid replication is not complete until some later stage.

The behavior of the meiotic chromosomes in response to X-irradiation is similar to that in postmeiotic mitosis. Crouse found chromatid bridges and free fragments at the first meiotic anaphase following irradiation of pachytene stages. Each of the two homologous chromosomes paired at pachytene consists of two chromatids, a stage comparable to prophase of mitosis. Irradiation of the first meiotic metaphase resulted in half-chromatid bridges at anaphase, indicating the four-partite nature of each of the two homologous chromosomes. Meiotic metaphase is the stage of chromosome duplication as detected by X-ray breakage and is comparable to prometaphase in mitosis. We have also obtained half-chromatid bridges by irradiating meiotic chromosomes at, or just prior to, the first meiotic metaphase in *Tradescantia*. Aberrations induced at prometaphase and metaphase of root-tip chromosomes of *Tradescantia* also consist almost exclusively of half-chromatid bridges, indicating the similarity of metaphase chromosomes in the somatic, meiotic, and haploid cells.

DNA synthesis during meiosis shows little relation to chromosome duplication as indicated by the nature of X-ray-induced aberrations. The indirect cytological and radiation evidence indicates that the leptotene chromosome is comparable with the resting-stage somatic chromosome, that the pachytene stage in meiosis is comparable to prophase in mitosis, and that the first meiotic metaphase is comparable to prometaphase in mitosis. Autoradiographs of meiotic chromosomes of *Tradescantia* show an increase of DNA at pre-leptotene but no further increase during the entire meiotic cycle, according to Taylor.[8] Plaut,[10] working with *Lilium*, found no evidence from P^{32} autoradiographs that any new DNA was incorporated in the meiotic chromosomes between leptotene and the end of the two meiotic divisions. Sparrow,[11] however, found an increase in DNA synthesis during pachytene and diplotene in *Trillium*—stages of meiosis comparable to prophase in mitosis.

If chromosome replication involves identical developmental stages of both sister chromatids, it is difficult to explain why only one is broken by irradiation at metaphase. If, however, the original chromatid produces the daughter chromatid in a sequence of chemical changes, the newly found chromatid might be particularly resistant or susceptible to radiation damage at certain stages of development. Perhaps the evidence from DNA synthesis might be of some aid in the analysis of chromosome duplication, but the differences in magnitude between molecular synthesis and gross chromosome duplication would appear to be too great to justify a direct comparison.

If, as many competent cytologists maintain,[12] the somatic anaphase chromosome is four-partite, any relationship between chromosome duplication and breakage by

X-rays must be secondary. It is possible that chromosome duplication occurs one cell generation before the two components can be differentiated by the induction of half-chromatid breaks and two cell generations before they separate at anaphase. Perhaps the actual duplication could be pushed back even further in chromosome synthesis, until DNA duplication is synchronized with chromosome duplication. If so, we would have to consider the chromosome as a multistranded unit at all stages in the nuclear cycle, emerging as a discrete unit only after many replications at the molecular level.

Summary.—In response to X-ray-induced aberrations, the *Tradescantia* microspore chromosomes react as single threads when irradiated during the resting stage, as double threads when rayed at prophase, and as four-partite strands when rayed at prometaphase and metaphase. The breaking of only one of the half-chromatids at metaphase, but of both at the resting stage, may be related to the nature of the half-chromatids at the time of duplication. The time of chromosome duplication as indicated by the types of X-ray-induced aberrations is compared with autoradiographs of DNA synthesis in microspore development and in the meiotic cycle.

* This work was supported in part by contract with the United States Atomic Energy Commission.

[1] C. P. Swanson, *J. Gen. Physiol.*, **26**, 485, 1943.
[2] Helen V. Crouse, *Science*, **119**, 485, 1954.
[3] Karl Sax and E. V. Enzmann, these PROCEEDINGS, **25**, 397, 1939.
[4] Karl Sax, *Cold Spring Harbor Symposia Quant. Biol.*, **9**, 93, 1941.
[5] D. E. Lea, *Actions of Radiations on Living Cells* (Cambridge: The University Press, 1947).
[6] B. P. Kaufmann, *Botan. Rev.*, **14**, 57, 1948.
[7] H. Swift, *Intern. Rev. Cytol.*, **2**, 1, 1953.
[8] J. H. Taylor, *Exptl. Cell. Research*, **4**, 164, 1953.
[9] Alma Howard and S. R. Pelc, *Heredity*, **6**, 261, 1952.
[10] W. S. Plaut, *Hereditas*, **39**, 438, 1953.
[11] A. H. Sparrow, M. J. Moses, and R. Steele, *Brit. J. Radiol.*, **25**, 182, 1952.
[12] B. R. Nebel, *Botan. Rev.*, **5**, 563, 1939.

*BREAKAGE OF CHROMOSOMES IN TRADESCANTIA WITH A CALCIUM DEFICIENCY**

BY DALE STEFFENSEN

BIOLOGY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY, UPTON, LONG ISLAND, NEW YORK

"Spontaneous" chromosome breakage in *Tradescantia paludosa* increases when plants are grown in decreasing amounts of magnesium.[1] Since calcium and magnesium show certain biochemical similarities and are components of chromosomes and nucleoprotein,[2] it seemed of interest to determine the effects of a calcium deficiency on chromosome stability. The present paper deals with attempts to demonstrate the importance of calcium in the structure and stability of chromosomes, since the motivating hypothesis of this work states that bivalent cations fulfil a structural requirement within nucleoprotein.

Materials and Methods.—The cultural methods were the same as those described previously.[1] Clonal divisions from a single plant of *Tradescantia paludosa* (clone