NONRANDOM SEGREGATION OF SISTER CHROMATIDS IN VICIA FABA AND TRITICUM BOEOTICUM*

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Experiments measuring the segregation of genetic material[1-4] in bacteria have suggested a model[4] in which the unit of segregation is a single polynucleotide strand of the DNA double helix. According to this model a polynucleotide strand becomes *permanently* attached to the cell's segregation apparatus when it is first used as a template in DNA replication. Thus, upon completion of replication, the finished chromosome is attached to the segregation apparatus by the nucleotide strand which served as a template, while the newly synthesized strand is unattached.

The usefulness of such a model lies in its extrapolation to eukaryotic organisms in which the genetic information is contained in several chromosomes. A copy of each of these must be distributed into each daughter cell. To ensure this, an accurate indexing system must be available, capable of separating all the replicated chromosomes (each containing several centimeters of DNA) into two equivalent chromatid sets. The model proposed for bacteria does just this. It assumes that each chromosome contains a single DNA double helix[5] (i.e., is unineme), and proposes that prior to replication this is attached to a segregation apparatus via that one of the two nucleotide strands which had served as a template. When replication is initiated, the complementary polynucleotide strand is attached to a new segregation apparatus to which new polynucleotide strands from each of the other chromosomes are similarly attached. In this manner, each group of sister chromatids is maintained as a set. A prediction of this model is that the set of chromatids which contain a polynucleotide strand made during the preceding division cycle (parent templates) will be separated into a different cell from the set which contain polynucleotide strands made two division-cycles earlier (grandparent templates).

This prediction was borne out by autoradiographic studies on mammalian cells growing in tissue culture.[6]

The present experiments demonstrate a similar phenomenon in growing root tips of *Vicia faba* and *Triticum boeoticum*.

Materials and Methods.—Seeds of *Vicia faba* were germinated on wet filter paper until secondary roots could be observed. Seedlings were then placed in half-strength Hoagland's solution for two hours, after which they were transferred into half-strength Hoagland's solution[7] containing 3 μc/.2 μg/ml of thymidine.

After 20 hours (label), one or two of the secondary roots were removed and the seedlings were washed and placed in nonradioactive half-strength Hoagland's solution.

The remainder of the secondary roots were removed after 40 hours of further growth (chase). All of the secondary roots were washed after removal and then fixed in Carnoy solution for 1–5 hours. The tips were stained by the Feulgen procedure and squashes prepared. These were subsequently dipped in Kodak NTB-2 emulsion and, after an appropriate exposure, developed. Preparations were examined at a magnification of $\times 1250$. Seedlings of *Triticum* were tested in the

same way at 20°C except that they were labeled for 10 hours and then chased for 20.

Results.—According to the model proposed above, cells which are labeled for one generation with radioactive thymidine and then allowed to grow for another one or

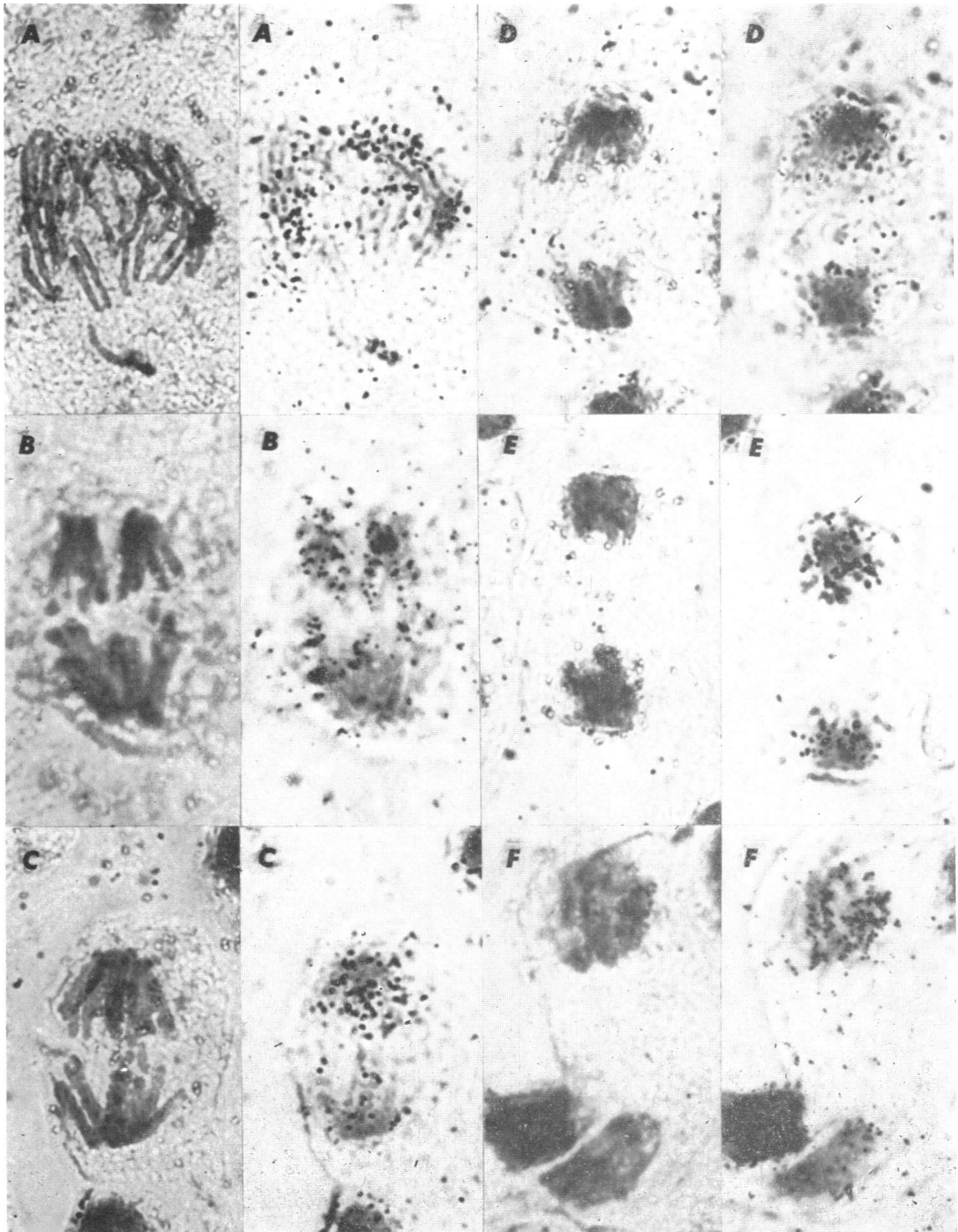

FIG. 1.—Photomicrographs of radioactive cells of *Vicia faba* in anaphase or telophase. Pictures were taken in the plane of the chromatid sets and of the film. Cells were taken from the experiment in Fig. 2*B*. Note the unequal distribution of label and the tendency for the less radioactive chromatid set to produce grains over material distal to the centromere.

two generations in nonradioactive medium should produce two sets of sister chromatids: those with radioactive and those with nonradioactive chromatids.

This may be measured by examining cells in anaphase or telophase. In such cells the immediate common ancestry of the two sets of chromatids is clearly defined.

In the first experiment, root tips of *Vicia faba* were examined which had been labeled with H^3-thymidine for approximately one generation (20 hr) and then grown for another two generations in nonradioactive medium. Figure 1 shows several examples of cells in anaphase or early telophase. It is obvious that the two sets of sister chromatids are not equally labeled.

Preparations of cells taken immediately after labeling, or after two further generations of growth in nonradioactive medium, were examined in anaphase and

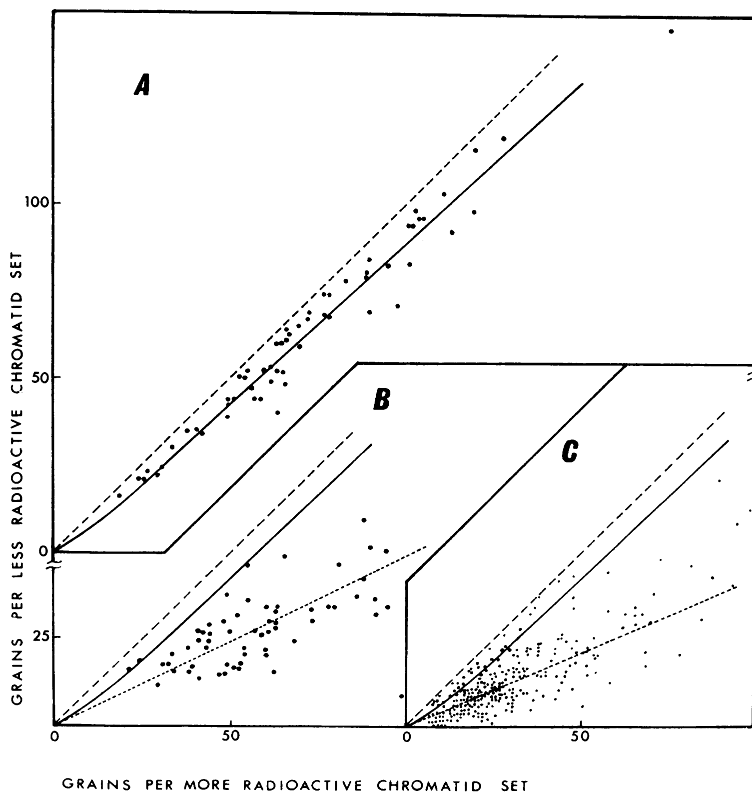

FIG. 2.—*Vicia faba* scatter diagrams in which each anaphase or telophase is located on the ordinate by the number of silver grains over the less radioactive chromatid set, and on the abscissa by the number of silver grains over the more radioactive chromatid set. Thus, no point can lie above the dashed line of slope 1, which represents the expected value for equal distribution of grains. The thin solid line represents the standard deviation (Poisson distribution) expected if the grains are the result of radioactivity emenating from equally radioactive sets of daughter chromatids. (*A*) After 20 hr of labeling with radioactive thymidine, 72% of the points lie between the dashed and the solid line; (*B*) after 20 hr of labeling with radioactive thymidine and 40 hr growth in nonradioactive medium. The thin dotted line represents the median for the distribution of grains between chromatid sets. Only samples with 30 grains or more were counted. (*C*) An experiment like (*B*) except that all anaphase or telophase figures with 5 or more grains were counted.

the number of grains in each set of chromatids counted. These data are shown in Figures 2 and 3. Immediately after labeling (Figs. 2*A* and 3*A*), the radioactivity is almost equally distributed between the two sets of chromatids—as predicted by the earlier data of Taylor and others.[5, 8] Thus, 70 per cent of the points in Figure 2*A* lie between the value expected for equal distribution and its standard deviation (calculated from a Poisson distribution). This equal distribution occurs despite a large variation in the extent to which the cells are labeled.

The data in Figures 2*A* and 3*A* demonstrate that the method of preparing cells for radioautography does not give rise to artifacts which could cause one set of chromatids to appear appreciably less radioactive than the other (such as different amounts of absorption over different portions of the cell).

In analyzing the distribution of radioactivity between sets of chromatids from cells which had been grown for one generation in radioactive medium and two in nonradioactive medium, we initially excluded all cells with less than 25 grains. In so doing, we focused attention on the first division after one generation of growth in nonradioactive medium and tended to exclude cells which received only pieces of radioactive chromosomes as a result of sister-chromatid exchange. The results are shown in Figures 2*B* and 3*B*. The results of another experiment, in which all of the labeled anaphase and telophase figures were counted, are shown in Figures 2*C* and 3*C*.

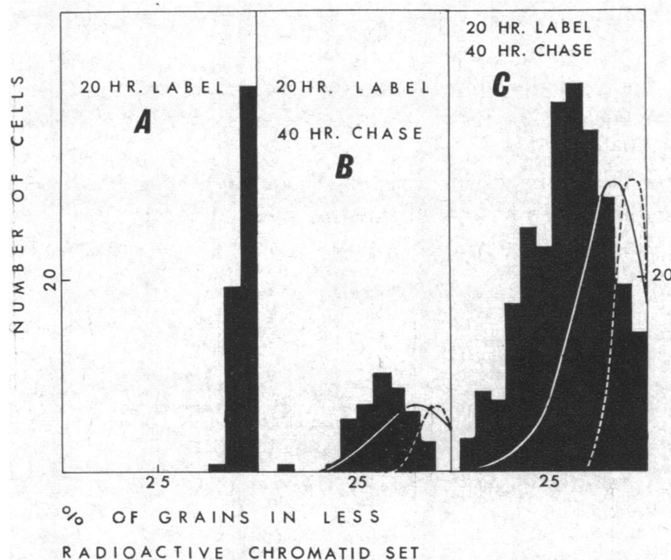

FIG. 3.—A representation of the distributions in Fig. 2, based on the per cent of grains in the less radioactive chromatid set of each cell. The distributions do not distinguish between cells with high and low amounts of radioactivity. The solid line indicates the random distribution of 12 radioactive chromatids calculated from the terms of a binomial expansion, assuming no sister-chromatid exchange. The dashed line is the distribution expected for the random distribution of 12 radioactive chromatids in which sister-chromatid exchange[11] results in the redistribution of radioactive material such that on the average 70% of the original chromatid material is not exchanged.

It is evident that the two sets of chromatids in such cells are not equally labeled; instead, one set is about twice as radioactive as the other. This result is not a reflection of a few highly radioactive cells. The distribution in Figure 3*B* is symmetrical, since the median and the mean are almost the same (see Table 1). The distribution in Figure 3*C* is similar but somewhat broader, reflecting a possible effect of sister-chromatid exchange. The distributions in Figures 3*B* and *C* are quite different from the distribution predicted for the random segregation of 12 radioactive chromosomes. This was calculated from a binomial expansion assuming each of the chromosomes is equally radioactive and that no sister-chromatid exchange occurs (*solid line* in Figs. 3*B* and *C*).

Sister-chromatid exchange[9-11] will introduce radioactivity into the unlabeled chromatid set. That this occurs is indicated in several of the anaphases in Figure 1 in which the label in the less radioactive chromatid set is concentrated in regions of the chromatids distal to the centromere.

Peacock[11] has estimated the frequency of sister-chromatid exchange as 0.7 for each of the telocentric S chromosomes of *Vicia faba* and 1.2 for the metacentric M chromosome, or an average of about 0.66 per long chromosome arm. These are the exchanges accumulated in two replication cycles. If such exchanges occur with equal frequency throughout the length of the chromosome, we may expect that on the average they will result in the exchange of 25–33 per cent of the material between chromatid sets (depending on the number of double exchanges which occur). When the binomial distribution in Figures 3*B* and *C* is corrected for such exchange by assuming that on the average each chromosome is distributed in such a way that 70 per cent of its radioactivity goes to one chromatid set and 30 per cent to the other, the distribution shown by the dashed line is obtained. Clearly, it does not fit the observed distribution.

On the other hand, the effect of sister-chromatid exchange agrees well with the observed distribution if we assume that radioactive chromatids tend to segregate as a set separating from nonradioactive ones. From these data, we have concluded

TABLE 1

SUMMARY OF THE DATA PRESENTED IN FIGURES 2–5

Material	Treatment	Radio-auto-graph exposure (days)	No. anaphases counted	Mean no. gns per anaphase	Mean no.* gns/less radioactive chromatid set	Mean of %* gns/less radioactive chromatid set	Median of % gns/less radioactive chromatid set
Vicia faba (Fig. 2*A*)	20-Hr label	15	64	136.4	64.2 (47%)	46.7	47.5
Vicia faba (Fig. 2*B*)	20-Hr label, 40-hr chase	15	70	84.6	27.6 (32.6%)	32.3	31
Vicia faba (Fig. 2*C*)	20-Hr label, 40-hr chase	10	257	43.8	13.9 (31.7%)	29.9	30
Triticum boeoticum diploid	10-Hr label, 20-hr chase	5	53	51.9	16.9 (32.6%)	32.2	31
Triticum aestivum hexaploid	10-Hr label, 20-hr chase	5	38	79.7	32.6 (40.9%)	39.6	39

The high average number of grains (gns) per anaphase or the data in Fig. 2*B* as compared to Fig. 2*A* reflects the fact that the less radioactive cells were not included in the distribution.

The mean number of grains/less radioactive chromatid set is the average of all the ordinate values for the points in Figs. 2 or 4. The values of the mean of the per cent grains in the less radioactive chromatid set are the means of the distributions in Figs. 3 and 5.

* The mean number of grains (gns) per less radioactive chromatid set is strongly influenced by cells with high grain counts. The mean of the per cent of grains (gns) in the less radioactive chromatid set is influenced to an equal extent by each of the cells in the distribution whether they are strongly or weakly radioactive.

that segregation separates chromatids containing polynucleotide strands made during the previous generation (radioactive) from chromatids made two generations previously (nonradioactive).

Figures 4 and 5 present preliminary results on studies with the wheats *Triticum boeoticum* (diploid) and *Triticum aestivum* (hexaploid). Previous studies with mammalian cells indicated that a polyploid strain (HeLa) did not show the clear nonrandom segregation of sister chromatids seen in diploid cells. The studies on wheat were initiated to investigate this problem. We found that almost all of the cells in root tips of wheat grown at 20°C were labeled within 12 hours. Seedlings were accordingly labeled for 10 hours and then grown in nonradioactive medium for 20. Because cells in anaphase or telophase were rare, the results presented were obtained from several plants.

It may be seen that sister-chromatid segregation in the diploid variety of *Triticum* is, again, nonrandom. Indeed, the values obtained are almost indistinguishable from those for *Vicia*. On the other hand, the hexaploid variety displays a much more nearly random pattern of sister-chromatid segregation, which could be composed of two cell populations: those in which segregation proceeds in a non-

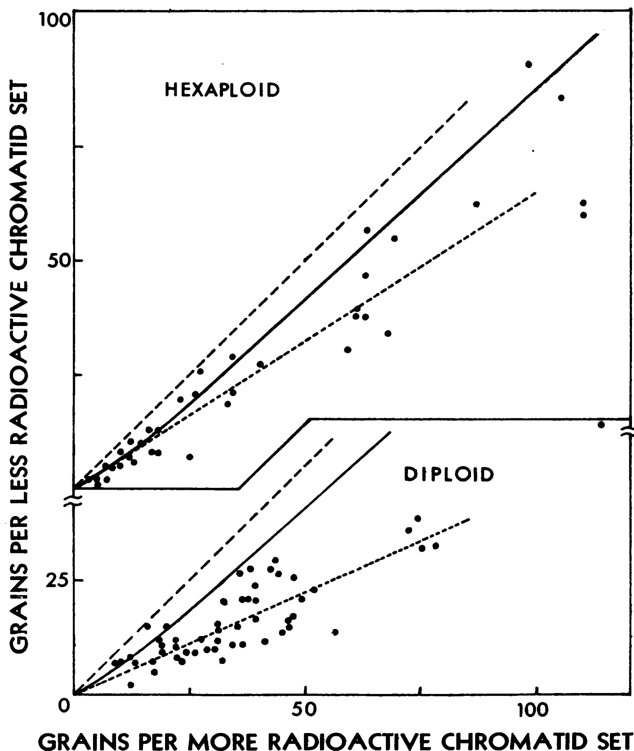

FIG. 4.—Scatter diagrams (as in Fig. 2) for diploid and hexaploid wheats (*Triticum boeoticum* and *Triticum aestivum*). Both types were labeled with radioactive thymidine for 10 hr and grown in nonradioactive medium for 20. As before, the expectation for equal distribution and its standard deviation are given, together with the median for the observed distribution of grains between chromatid sets.

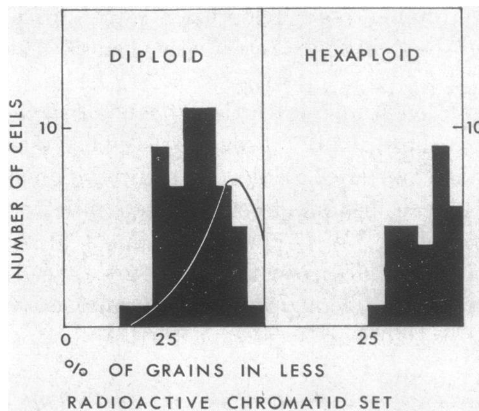

FIG. 5.—Representation of the distributions in Fig. 4 as described in the legend to Fig. 3. The solid line indicates the random distribution of 14 radioactive chromatids calculated from the terms of a binomial expansion, assuming no sister-chromatid exchange.

random fashion, similar to *Vicia*, and those in which sister-chromatid segregation is almost random.

This appearance of two types of segregation patterns appears to be independent of the extent to which cells have incorporated label. Moreover, both types of cells were observed in preparations from a single plant. The results of these experiments also are summarized in Table 1.

Discussion.—The data presented affirm our previous conclusion that sister-chromatid segregation is not random. As in bacterial[4] and mammalian cell[6] systems, sister-chromatid segregation in plants tends to maintain a group of chromatids which contain a template nucleotide strand synthesized in a particular previous generation. Redistribution of this material occurs as a result of sister-chromatid exchange. In addition to extending the generality of our previous conclusions, the plant system adds two other factors: (*a*) nonrandom segregation is *not* dependent on the existence of a visible centriolar structure; (*b*) nonrandom segregation may be observed in an *in vivo* system and is therefore not an artifact of a cell culture system.

The observations on *Triticum aestivum* are based on a small sample. The preliminary finding that polyploidy randomizes sister-chromatid segregation is consistent with our previous finding in HeLa cells.[12] Because HeLa cells are aneuploid, it is difficult to correlate segregation patterns with changes in ploidy. This is not the case for wheat.

In particular, these experiments raise the possibility that in hexaploid organisms, each diploid set of chromosomes may behave independently during segregation. This would give rise to two populations of cells: one in which two of the three sets of radioactive chromatids segregate together, another in which all three segregate together. However, more extensive experiments must be carried out under various growth conditions before this hypothesis can be established.

In contrast to the mammalian system, segregation in *Triticum* is amenable to detailed genetic and cytogenetic analysis.[13, 14] Thus, it may be possible in future studies to correlate nonrandom sister-chromatid segregation with chromosome behavior at meiosis, such as the ability of homologues and homeologues to pair.[13]

Summary.—Root tips of *Vicia faba* were labeled with H^3-thymidine for 20 hours and then grown in nonradioactive medium for 40. Examination of sister-chromatid sets at anaphase and early telophase demonstrated a tendency of radioactive chro-

matids to segregate together separating from nonradioactive chromatids. The radioactive material found in the less radioactive set of chromatids corresponds to the amount expected on the basis of sister-chromatid exchange. A diploid strain of *Triticum* shows a similar segregation pattern, whereas a hexaploid strain does not.

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