SISTER CHROMATID SEGREGATION DURING MITOSIS IN POLYPLOID WHEAT

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The chromosomes of eukaryotes replicate in a semi-conservative manner (Taylor, Woods and Hughes 1957; Prescott and Bender 1963). Utilizing this observation, we have studied the segregation of sister chromatids during mitosis of plant and animal cells (Lark, Consigli and Minocha 1966; Lark 1967). Autoradiographic measurements of segregation at the second mitosis following incorporation of radioactive label indicated that sister chromatids were not always segregated randomly. Instead, chromatids synthesized during any particular generation tended to remain together and to segregate as a group. These experiments were instigated by measurements of DNA synthesis and segregation in bacteria (Lark 1966). Their rationale has been discussed in detail elsewhere (Lark 1969).

The experiments with eukaryotes indicated that different systems might segregate in a more or less random manner depending on the ploidy of the system (Lark, Consigli and Minocha 1966; Lark 1967). Recently, the interpretation of these experiments has been challenged by results obtained using a different experimental approach (Heddle, Wolff, Whissell and Cleaver 1967; Cuevos-Sosa 1968).

These authors have used a different technique to study sister chromatid segregation. Whereas we have measured the amount of radioactivity (number of silver grains) associated with a chromatid set, they have measured the number of labelled chromosomes which remain associated during the second and third cycles of mitosis and division which follow radioactive labeling. A common organism, Vicia faba, was studied by Heddle et al. (1967) and by ourselves. They observed a random distribution of radioactive chromosomes in second and third mitosis, we observed a non-random distribution of radioactivity among sister-chromatid sets. Heddle et al. (1967) explained the difference by attributing our distribution to the random segregation of a few radioactive chromosomes (in Vicia this would be six or less at the second or third mitosis after growth) which could lead to an asymmetric distribution of radioactive label. On the other hand, sister chromatid exchanges at the kinetochore would obscure a non-random distribution which might have existed in their studies and no data were presented to compare the frequency with which one or both arms of the metacentric chromosome were labeled. Prescott and Bender (1963) have estimated that the frequency of sister chromatid exchange at the kinetochore will account for 25% of all sister chromatid exchanges.

Nevertheless, the results of Heddle et al. (1957) and of Cuevas-Sosa (1968) indicated that non-random sister chromatid segregation was probably not a necessary part of the mechanism whereby chromosomes are assorted into daughter cells.

The experiments described below were designed to obtain more information on this problem by answering three questions:

A) Does non-random sister chromatid segregation occur in polyploid organisms?

B) Is it under genetic control?

C) Is it a necessary part of the mechanism which insures correct distribution of sister chromatids into daughter cells?

For our experiments, we chose hexaploid wheat. The genetics of this organism is well established (Sears 1966; Sears and Okamoto 1956). It possesses three homeologous sets of chromosomes, each such diploid set containing seven chromosome pairs. Strains are available in which certain chromosomes are lacking and in which others are present in extra dosage (nullisomic-tetrasomic) (Sears 1966).

One of the chromosomes, 5B, has been demonstrated to control pairing and segregation at meiosis (Sears and Okamoto 1958; Riley and Chapman 1958; Riley 1960; Riley and Kempenna 1963; Feldman 1966) and strains lacking 5B or containing extra doses of it are available.

Previous experiments (Lark 1967) had demonstrated that germinating seedlings of diploid and hexaploid wheat could be labeled with tritiated thymidine. Segregation was followed by autoradiography of sister sets of chromatids by measurement of the relative amount of radioactivity in anaphase figures fixed during the second or third mitosis after incorporation of radioactive label. Whereas the diploid material displayed a non-random segregation pattern, preliminary experiments with the hexaploid plants indicated a different, more random, pattern.

The experiments described below were carried out with hexaploid and tetraploid plants of established genetic constitution.

**MATERIALS AND METHODS**

Strains of hexaploid wheat were obtained from Dr. E. R. Sears. Tetraploid strains were obtained from Drs. Tsunewaki and Feldman. Table 1 lists the strains used, their source and their genetic composition.

In all but two experiments, the seeds were germinated and grown on wet filter paper at room temperature 22-24°C. Immediately after germination (when the roots were about 1 mm long), the seedlings were placed in a Petri dish containing 5 ml of a solution of 3H thymidine (New England Nuclear Corporation) at a specific activity of 3 μC/μg/ml. After 12 hr, the seedlings (whose roots were now about 1 cm long) were washed and transferred to fresh Petri dishes containing wetted filter paper. One hour later, one of the three root tips was removed, washed and fixed in cold 50% acetic acid. These were used to study the first mitoses after incorporation of radioactive label. The rest of the plant was allowed to grow for 24 hr in non-radioactive medium, after which the remaining root tips were removed and fixed in 50% glacial acetic acid. These served as a source of second or third generation mitosis after incorporation of radioactive label.

The fixed root tips were stained with Feulgen, squashed and fixed on microscope slides (Lark 1967). These were dipped in Kodak NTB-2 emulsion and after suitable exposure developed.
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TABLE 1
List of strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Genetic composition</th>
</tr>
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<tbody>
<tr>
<td><em>Triticum aestivum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Var. Chinese spring</td>
<td>E. R. Sears</td>
<td>2-5A; 2-5B; 2-5D</td>
</tr>
<tr>
<td>Var. Nulli 5B-tetra 5D</td>
<td>E. R. Sears</td>
<td>2-5A; 0-5B; 4-5D</td>
</tr>
<tr>
<td>Var. Nulli 5D-tetra 5B</td>
<td>E. R. Sears</td>
<td>2-5A; 4-5B; 0-5D</td>
</tr>
<tr>
<td><em>T. boeticum</em> (tetraploid)</td>
<td>K. Tsunewaki</td>
<td>AAAA</td>
</tr>
<tr>
<td><em>T. aegilopoides</em></td>
<td>M. Feldman</td>
<td>AADD</td>
</tr>
<tr>
<td>- <em>Aegilops squarrosa</em> (amphidiploid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. durum</em> var.</td>
<td>K. Tsunewaki</td>
<td>AABB</td>
</tr>
<tr>
<td>reichenbachii</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. monococcum-</em></td>
<td>M. Feldman</td>
<td>AABB</td>
</tr>
<tr>
<td><em>Ae. bicornis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(amphidiploid)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The three strains of *T. aestivum* are isogenic for all but one of the A, B and D chromosomes. The composition of chromosomes of homeologous group 5 is noted.

Anaphase and telophase figures were located and the number of grains over each chromatid set counted in a Wild M-20 microscope at a magnification of 1500×.

In two experiments, seeds were germinated and grown at 34°C. In these cases, label was incorporated for 8 hr and cells were subsequently grown in non-radioactive medium for 16 hr.

RESULTS

In the first series of experiments, segregation of chromatids was studied in hexaploid *T. aestivum* plants containing different doses of chromosomes 5B, or 5D. The strains studied were: wild type (2-5D; 2-5B); tetra 5D-nulli 5B, (4-5D; 0-5B); and nulli 5D-tetra 5B (0-5D; 4-5B).

The data from these experiments are shown in Figures 1–6. In every cell the two chromatid sets are either equally radioactive or one set is more radioactive than the other. The data initially are presented as a scatter plot in which each point represents a single anaphase; the ordinate gives the number of silver grains over the less radioactive chromatid set while the abscissa gives the number of grains over the more radioactive. As a result of this method of presentation, each point represents a single cell and will lie on, or below a 45° line which intercepts the origin. The closer the points are to this line, the more nearly equal the radioactivity between the two chromatid sets. Points lying far below this line characterize anaphase figures in which one chromatid set is much less radioactive than the other. An advantage of this type of graph is that the raw data can be presented directly and that highly radioactive cells are especially noticeable. The larger number of silver grains make such cells statistically more significant.

Figures 1, 2 and 3 (●) present the distribution of radioactivity between chromatid sets measured in cell samples immediately after radioactive labeling. In every case, the distribution of points lies close to the line predicted if the radio-
activity of the two sets were equal. Such an equal distribution of radioactivity is predicted by a semi-conservative mechanism of replication, according to which both sister chromatids should be equally labeled after replication. Points which scatter below the line, do so in a manner consistent with the statistical deviation expected between samples of equal radioactivity. (The probability of observing a certain number of silver grains for a chromatid set of fixed radioactivity will correspond to a Poisson distribution.)

The amount of radioactivity incorporated into individual cells (the sum of the $x$ and $y$ coordinates) can be seen to vary. This variation also was observed between individual cells from a single root tip. In all probability, it represents variation between individual cells in the amount of endogenous non-radioactive thymine available for DNA synthesis. It is of passing interest that less variation was ob-
served in the seedlings of (0-5B; 4-5D) plants (see also Figure 8 below). The genetic and physiological basis of this difference has not been investigated.

Two conclusions may be derived from the data in Figures 1–3: (A) Determination of radioactivity from autoradiographs of labeled anaphase or telophase figures is not biased by unequal self adsorption over one of the two sister chromatid sets, since the numbers of grains over both sets of a pair are approximately equal. (B) There is no observable effect of genetic composition on the distribution of radioactivity between sister chromatid sets at the first mitosis after labeling.

When labeled roots are allowed to grow in non-radioactive medium, non-radioactive chromatids are again produced according to the predictions of semi-conservative replication. Their distribution into daughter cells is quite different.
FIGURE 3.—Distribution of radioactivity between sister chromatid sets of *T. aestivum* nulli 5D-tetra 5B (0-5D; 4-5B). (●) Samples taken 1 hr after incorporation of radioactive label. (○) Samples taken 2½ hr after incorporation of radioactive label. Radioautographs were exposed for six days.

For explanation of graph see Figure 1.

depending on the genetic composition of the plant examined. Figures 3 (○) and 4 show the results of two separate experiments of this type in which (0-5B; 4-5D) seeds were studied. It is clear that the majority of cells contain anaphase or telophase figures in which the two sister chromatid sets are almost equally radioactive. Since these chromatid sets (Figure 3 (○)) are uniformly lower in radioactivity than those from the first mitosis after labeling (Figure 3 (●)) (note the difference in exposure of the autoradiographs), it seems safe to conclude that one or more cell division has occurred since radioactive labeling. Thus, unlabeled and labeled sister chromatids must be distributed with equal probability into daughter chromatid sets.

This is not the case for cells from plants with lower doses of the 5D and higher doses of the 5B chromosomes. Figures 5 and 6 present results from chase experiments with plants of genetic composition: (2-5D; 2-5B) and (0-5D; 4-5B), respectively. It can be seen that with increasing 5B and decreasing 5D dosage the
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Figure 4.—Distribution of radioactivity between sister chromatid sets of *T. aestivum* nulli 5B- tetra 5D (0-5B; 4-5D) grown at 20°C. Samples taken 24 hr after incorporation of radioactive label. Seedlings labeled as in Figure 3. (O) Radioautographs exposed for 6 days. (●) Radioautographs exposed for 4 days.

For explanation of graph see Figure 1.

distribution of radioactivity between sister chromatid sets becomes progressively more asymmetric, indicating an increasing tendency toward a non-random distribution of the newly synthesized non-radioactive sister chromatids.

This is most clearly seen in Figure 7 in which the distribution of radioactivity between sister chromatid sets is presented as the frequency of sets with a given percent of radioactive material (silver grains) in the less radioactive chromatid set from each mitotic figure. In this graph, chromatid sets which contain close to 50% of the radioactivity are derived from cells in which the two sets are equally labeled. Asymmetric distribution of non-radioactive chromatids will force the distribution to the left. It is clear that such a shift occurs as the dosage of 5D decreases and that of 5B increases.

It has been observed that the effect of chromosome 5B on pairing at meiosis is enhanced by growth at higher temperature (Feldman 1966). A similar effect of temperature was apparent in our experiments. Figures 8, 9 and 10 present data from experiments in which seeds were germinated, labeled and chased at 34°C. In cells from (0-5B; 4-5D) plants labeled and unlabeled chromatids are distrib-
Figure 5.—Distribution of radioactivity between sister chromatid sets of *T. aestivum* (2-5B; 2-5D) grown at 20°C. Samples taken 24 hr after incorporation of radioactive label. Seedlings labeled as in Figure 2. For explanation of graph see Figure 1. Radioautographs exposed for six days.

Grains per less radioactive chromatid set

Grains per more radioactive chromatid set

Figure 5.—Distribution of radioactivity between sister chromatid sets of *T. aestivum* (2-5B; 2-5D) grown at 20°C. Samples taken 24 hr after incorporation of radioactive label. Seedlings labeled as in Figure 2. For explanation of graph see Figure 1. Radioautographs exposed for six days.

uted with equal probability into daughter cells, whereas those from (4-5B; 0-5D) plants tend to be distributed in groups assorted according to the origin of their synthesis—labeled chromatids into one set, unlabeled ones into the other.

It is interesting to note that one (4-5B; 0-5D) seedling yielded eight anaphase figures, all of which were of a random pattern. It seems likely that this seed was the result of stray pollination (the plants from which the seed was derived were not "bagged" (SEARS, personal communication)) and was not actually (4-5B; 0-5D).

The experiments in Figures 1–10 do not allow us to determine whether the effects observed are the result of the presence or absence of the 5B or of the 5D chromosomes. To test this, tetraploids were examined. The following combinations were investigated: AAAA, (4-5A); AADD, (2-5A; 2-5D); and AABB, (2-5A; 2-5B).

The results are presented in Figures 11–15. It can be seen that segregation of sister chromatids is non-random for all of the combinations except AADD, (2-5A; 2-5D). The grain distributions for the control samples (first mitosis after label-
**Figure 6.** Distribution of radioactivity between sister chromatid sets of *T. aestivum* nulli 5D-tetra 5B (0-5D; 4-5B) grown at 20°C. Samples taken 24 hr after incorporation of radioactive label. Seedlings labeled as in Figure 1. Radioautographs exposed for six days.

**Figure 7.** Effect of gene dosage on the distribution of radioactivity between sister chromatid sets. Data from Figures 4-6 are compared for plants with nulli 5D-tetra 5B, wild type and nulli 5B-tetra 5D grown at 20°C. Histograms of the frequency of cells in which the indicated percentages of grains were observed in the less radioactive chromatid set forming cell. Deviation to the left of 50% indicates the degree of asymmetry. The dashed curve represents the expected binomial distribution for random segregation.
DISCUSSION

At the first metaphase after incorporation of radioactive thymine, sets of chromatids are equally radioactive. This result agrees with previous observations that the chromosomes of eukaryotes replicate semiconservatively (Taylor, Woods and Hughes (1957); Prescott and Bender (1963)). In some of the strains of
wheat which were studied, unequal distributions of radioactivity were observed in metaphase figures following growth in non-radioactive medium. This could occur if sister chromatids were distributed at random into daughter cells but were unequally labeled. However, to explain the results with the nulli 5D-tetra 5B strain it would be necessary for the majority of the radioactive label to be concentrated in a few chromosomes. We have not observed such a difference although our preparations would permit us to detect only large differences in the level of radioactivity. We prefer to interpret the data in terms of the non-random segregation of sister chromatids each of which we assume to be equally labelled immediately after incorporation of radioactivity. Granted this assumption, our data allow us to draw the following conclusions:

A) Non-random segregation of sister chromatids can occur in polyploid plants. This is demonstrated by the asymmetric distribution of radioactive chromatids observed in nulli 5D-tetra 5B seedlings (Figure 6) following growth in non-radioactive medium after labeling. Since such plants contain 42 chromatids in each sister chromatid set, it is impossible to ascribe this degree of asymmetry to the random assortment of labeled chromatids according to a binomial distribution.

**Figure 9.**—Distribution of radioactivity between sister chromatid sets of *T. aestivum* nulli 5D-tetra 5B (0-5D; 4-5B) at 34°C. Samples taken 16 hr after incorporation of radioactive label. Radioautographs exposed for six days. Samples labeled as in Figure 1 (O).

For explanation of graph see Figure 1.
FIGURE 10.—Comparison of sister chromatid segregation in nulli 5B-tetra 5D and nulli 5D-tetra 5B plants grown at 34°C. Taken from data in figures 8 and 9. For explanation of the figure, see Figure 7. The dashed histogram represents points in parentheses from Figure 9.

FIGURE 11.—Distribution of radioactivity between sister chromatid sets of T. boeticum autotetraploid (AAAA). Seedlings were grown at 20°C and samples were taken 24 hr after incorporation of radioactive label. Radioautographs were exposed for 14 days.
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FIGURE 12.—Distribution of radioactivity between sister chromatid sets of T. durum (AABB). Seedlings were grown at 20°C and samples were taken 24 hr after incorporation of radioactive label. Radioautographs were exposed for 19 days.

(This explanation had been advanced previously by HEDDLE et al. (1967) to interpret our previous observations on Vicia faba.)

B) Non-random segregation of sister chromatids is under genetic control. This is clearly seen in the comparison (Figures 7 and 10) between (0-5D; 4-5B), (2-5D; 2-5B) and (4-5D; 0-5B) plants.

It was possible that an increasing probability of sister chromatid exchange was responsible for the observed differences in the distribution of radioactivity between sister chromatid sets. However, preliminary experiments (KIMBER and LARK, unpublished observation) have indicated that sister chromatid exchange occurs to the same degree in root tips of nulli 5B-tetra 5D and nulli 5D-tetra 5B seedlings.

Since the T. aestivum strains were isogenic for chromosomes other than 5B, we have concluded that segregation is controlled by information on the 5B or 5D chromosomes. Moreover, this control would appear to reflect the gene dosage, since non-random segregation is observable but less frequent in (2-5B; 2-5D) plants.

The details of this genetic control are clarified to some extent by the results from the tetraploid plants. (It must be cautioned, however, that these plants were not isogenic. It is always possible, therefore, that other genetic factors are influencing our observations.) AAAA as well as AABB, (2-5A; 2-5B) plants exhibit non-random segregation. Since two doses of chromosome 5A were present in all of the hexaploid plants, we must conclude that non-random segregation is a normal state of the plant which is not dependent on the 5B chromosome.
The random segregation of sister chromatids in tetra 5D-nulli 5B and in AADD, (2–5A; 2–5D) plants must, therefore, result from an effect of chromosome 5D. This could occur through a direct effect of 5D on the physiology of mitosis, or through an indirect effect, e.g., if 5D acted as a suppressor of the genes controlling non-random segregation.

C) Non-random sister chromatid segregation is not necessary in order to segregate chromosomes properly into daughter cells. This follows from the observation that nulli 5B-tetra 5D seedlings exhibit random sister chromatid segregation, yet such seedlings grow into plants in which each cell has its proper assortment of chromosomes (SEARS 1966). It follows, therefore, that chromatids of a set need not remain permanently attached to a common nuclear structure in order for proper segregation to occur.

Two explanations of non-random mitotic segregation can be considered. It is possible that individual chromatids are permanently attached to a nuclear structure composed of sub-units which are dissociated and subsequently re-assembled during each mitotic cycle (FELDMAN, MELLO-SAMPAYO and SEARS 1966; LARK 1967). The relationship of the individual attachment units to each other could be preserved to that extent that the total structure remains intact. This, in turn, could be determined by those genes which control the structure or synthesis of...
Figure 14.—Distribution of radioactivity between sister chromatid sets of *T. aegilopoides—Aegilops squarrosa* amphidiploid (AADD). Radioautographs were exposed for 14 days.

Figure 15.—Comparison of sister chromatid segregation in AAAA, AABB, and amphidiploid AADD and AABB plants. Taken from data in Figures 11–14 as well as additional, similar data, not included in those figures.
the sub-units of which the total structure is composed. According to such a model, genes on chromosome 5D would affect such sub-units. The nuclear membrane has been suggested as a candidate for the structure to which the chromatids might be attached (LARK 1967) and some evidence for such attachment has been obtained (COMINGS and KAKEFUDA 1968).

Alternatively, the pre-orientation of chromatids for mitotic segregation could occur through failure of a spindle apparatus to completely dissolve, thus preserving the relative orientation of a chromatid set from one division cycle to the next (R. EGEL, personal communication). This possibility is supported by the observation that colchicine can disrupt the pre-meiotic association of chromosomes in hexaploid wheat (DRISCOLL, DARVEY and BARBER 1967). This finding suggests that the existence of some pre-meiotic microtubular structure (BORISY and TAYLOR 1967a, 1967b) may influence the course of subsequent segregation (TAYLOR 1965).

Comparative biochemical and ultra-structural studies of the nuclear and mitotic structures in *Triticum aestivum* nulli 5D-tetra 5B and nulli 5B-tetra 5D strains should help to determine the mechanism responsible for non-random sister chromatid segregation.

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**SUMMARY**

Non-random sister chromatid segregation has been measured in wheat. Hexaploid and tetraploid strains, carrying different doses of chromosomes 5B or 5D have been studied. The randomness of segregation has been measured by autoradiography of cells from root tips labeled by growth in radioactive thymine and then grown in non-radioactive medium. Sister chromatids in cells from plants with a high dose of the 5D chromosome segregated at random. In all other plants segregation was non-random. Chromatids carrying DNA synthesized in the same generation tend to segregate together. The results led to the suggestion that non-random segregation was due to the persistence of microtubular structures in cells with low doses of the 5D chromosome. A product of the 5D chromosome could be effective in dissolving such structures.

**LITERATURE CITED**


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