Behaviour of Chromonemata in Mitosis. VII
A chromosome study by the artificial uncoiling method
of the chromonema spirals

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Pollen mother cells of *Tradescantia reflexa* were used as material, and the chromosomes in the heterotype division were observed. The original object of this investigation was as follows: In our previous paper (KUWADA and NAKAMURA, 1933) we have reported that in *Tradescantia reflexa* the two sister chromatid spirals are very closely appressed into each other in the I-metaphase, not only the major spirals, but also even the minor spirals, so as to form a seemingly double-stranded spiral. It seemed to me later, however, that this interpretation might be erroneous, because in other plants the sister chromatid spirals are clearly observed to lie side-by-side not being appressed into each other. If this is the case in our material too, the doubleness shown by the metaphase spirals must represent the doubleness of the chromonema of a chromatid, and not that due to the two sister chromatid spirals being closely appressed into each other. The doubleness of the metaphase spirals is observed undoubtedly and clearly, especially when the spirals are uncoiled artificially by means of alkaline solutions (OURA, 1936). Thus an important question from the view point of the problem of the longitudinal splitting of the chromosome must be determined. The question is which interpretation of this doubleness is correct—whether as representing doubleness of one of the sister chromatid spirals (the third split) or as due to a condition in which the two sister chromatid spirals are closely appressed into each other. The determination of this question was originally the chief object of the present investigation.

If the doubleness represents the doubleness of a chromatid spiral, the doubleness which is clearly observed in the metaphase should be observable also in the anaphase as clearly as in the metaphase, and if, on the other hand, it represents the two sister chromatid spirals no such a doubleness could expect to be observed in the anaphase, because in this stage the sister chromatids have been clearly separated. An ordinary method of direct observation will do for this purpose of critical observation, if the observation is carefully

made, but the use of the artificial uncoiling method of the chromonema spiral will make it easier and give a more decided result. The results we obtained with the latter method were as follows:—

The pollen mother cells were pretreated with a 1/100 mol NH_4OH for 2–3 minutes and then stained with acetocarmine. This method of using the 1/100 mol NH_4OH solution as a pretreating agent was first employed by SHINKE,¹⁾ and the preparations for the present investigation by this method were prepared by him at my request. The photographing was also done by him. I should like to take this opportunity of thanking him for his kind assistance on this side of the investigation.

In the present investigation only those cells were observed, in which the chromosomes are uncoiled solely from the major spirals. In these preparations the sister chromosome pairs in anaphase present the shape of a double V or an X, or nearly so, with the attachment point at the apex of the V's or the cross point of the X. The minor spirals remain intact and are visible more or less clearly in the four arms of the double V or the X. In the cell reproduced in Fig. 1, the anaphasic chromosome distribution remains, not severely disturbed, but in most cases the chromosomes are found so widely scattered throughout in the cell (Fig. 2), that it is difficult to state, without comparing with some such as those shown in Fig. 1, at which stage the chromosomes were before the treatment.

The double V shape of the sister chromosome pairs would show that the doubleness of the metaphasic spirals is due to the doubleness of the spiral strands themselves, if the chromosomes are telomitic; and that it is due to the two sister chromatid spirals being closely appressed into each other, if the chromosomes are atelomitic. According to KATO (1935) the spindle fibre attachment of the chromosomes is approximately median in our material except for some which appear to be subterminal. We must then conclude that the metaphasic doubleness of the spiral strands represents no doubleness of the strands themselves, but shows a condition in which the sister spirals are closely appressed into each other, and hence that the interpretation we have made in the previous paper is correct (comp. Fig. 3 with Text-fig. 3b in the previous paper, KUWADA and NAKAMURA, 1933).

This conclusion does not mean that the "third split" is not observable in the first division (cf. HUSKINS, 1937). In our material this split is clearly observed only in the second division. In the first division the two half-chromatid spirals are closely appressed into each other again, and the duality of the chromonemata is visible only

1) In press.

fragmentarily in places. A fuller discussion of the problem will be made at another opportunity in a general form, but I may say here that I am quite in agreement with the view of Prof. HUSKINS in this respect.

In the course of this investigation, Mr. SHINKE found a peculiar phenomenon which is worth mentioning. In Fig. 4, it is shown that the sister chromosome arms are twisted around each other to form relational coils. These must represent the major coils. In the artificial uncoiling they must have remained uncoiled, or, in other words, the arms must have formed relational coils before the uncoiling treatment. This relational condition between the arms was confirmed in the case shown in Fig. 5 (comp. *a* and *b* at different foci) by carefully manipulating the microscrew. In *Trillium* such a case would not be expected to occur. If in *Trillium* in which the chromosomes in the second division are of the double-coiled structure as in the first division and "the first anaphase chromosomes pass without material change of structure to the second metaphase" (HUSKINS and SMITH, 1935) such relational coils are formed between the sister chromatids, the chromosomes can not separate in the second division. In *Tradescantia*, on the other hand, this twisting around of the sister chromatids would afford no obstacle to separation in the second division, because in this plant the interkinesis exists in which the chromosome spirals are uncoiled or made loosely coiled, and in the second division the major spirals have been uncoiled. We may, then, presumably say that this peculiar phenomenon of twisting around of, or the formation of relational coils between, the sister chromatids may occur, but is not of a universal occurrence. It seems highly probable that this phenomenon is closely correlated to the condition in early prophase of the sister chromatid chromonemata being closely appressed to each other.

Our interpretation is as follows:— We assume that the major spirals are the spirals that are formed first in early prophase, and the minor spirals are those which are formed second in late prophase (KUWADA, 1935). Therefore, we may assume that the relational condition between the major spirals of the sister chromatid chromonemata arose at the time of the first coiling of the chromonemata. In the case where the sister chromatid chromonemata are closely appressed to each other, it seems likely that a common twisting as the antecedent of spiralization (KUWADA, 1937) may sometimes occur, thus resulting in the formation of a pair of relational spirals. This pair of relational spirals will develop into a pair of relational major spirals later when the second coiling forms the minor spirals.

Fig. 1. I-anaphase. Major coils are uncoiled. Minor coils are visible more clearly than in the uncoiled state of the major coils. Fig. 2. The same. In this figure the original distribution of the chromosomes is greatly altered. Fig. 3. I-metaphase showing how the sister chromatid spirals are appressed into each other. Fig. 4. showing sister chromosome arms twisting around each other. Fig. 5. The same at different foci, *a* and *b*. Note the chromosome parts indicated by arrows. Figs. 1-5 $\times 1850$.

The relational major spirals which we have observed in *Tradescantia* may be regarded as spirals formed in such a way.

There seems to be a certain question as to whether the so-called minor spirals are regular spirals or a wavy corrugation (cf. HUSKINS, 1937). This divergency in the form of the spirals seems not to be a problem of fundamental importance, if we assume that the mechanism by which the spiralization is realized is such as we have postulated in the previous paper (KUWADA, 1937). We assume first that the chromonema which has a certain twisted tendency is brought to a twisted condition by an anisotropic hydration, and secondly that when the condition in which the hydration is isotropic is reached, and consequently when the twisted chromonema is forced to untwist, the contraction power of the chromosome matrix which increasingly develops in amount prevents the chromonema from its direct untwisting, but forces it to a wavy or zig-zag corrugation. The chromonema in this condition is still striving to untwist, and this can be attained only by transforming the wavy corrugation into a spiral form. A most beautiful model experiment we find in the case of swelling of the silk fibroin threads illustrated in his Fig. 9 by OHARA (1933). He describes it: "Schon bei der Einspinnung der Seide gewinnt der Kokonfaden eine schwache Drehung. Dieser Drehung erscheint besonders deutlich bei der Zentralzone, wenn der Faden einer starken Quellung unterworfen wird, wodurch er sich stark verkürzt" In this figure the spiralization has been beautifully accomplished in the part of the thread on the left hand side of the figure and in the remaining part it has not, a compact corrugation being a prominent feature. If we assume that the transformation of the corrugation into the spiral is less easy in the case of spiralization within a spiral than in the case of a simple thread, we may understand why in the heterotype division the minor spiral may not be a regular spiral, but a wavy corrugation. According to this interpretation, the question of the spiral form, whether a regular spiral or a mere corrugation, is not a fundamental problem, but only a consequence of secondary importance resulting from the difference in the condition in which the spiralization takes place.

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