The Internal Mechanics of the Chromosomes, V. Relational coiling of chromatids at mitosis

by

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1. Introduction

ANYONE who is familiar with mitosis in organisms with large chromosomes is aware that the daughter chromatids do not always lie parallel, but sometimes coil round one another at metaphase as well as earlier. Illustrations, particularly the excellent drawings of Lewitsky, show this property clearly. But the significance of the direction of coiling and of its consistency has not hitherto been appreciated. Earlier figures, my own as well as other workers', are therefore unreliable as evidence in this respect.

This question now calls for attention. I have suggested in the first of these studies that all the spiral relationships shown by the chromosomes are mechanically interconnected. Koller and I (1935) have given some evidence in the later studies for believing that the relic spirals at the prophase of mitosis and meiosis and the relational spiral of the prophase of meiosis are consistent with one another and are derived from the action of changes in an underlying molecular spiral which determines the major and minor spirals of the contracted metaphase chromosome.

I have also suggested that the coiling of chromatids at metaphase of mitosis (unlike that at prophase of meiosis) cannot be derived from the coiling of the previous division, since first it cannot occur in ring chromosomes, and secondly it can arise at the second meiotic division where the chromatids have coiled separately (1936a). It must be a product therefore of the new changes determining the spiralisation of the new division. And since it is an optional product it need not be directly related to the six obligatory types of spiral relationship that I have described.

The object of the present study is to find out whether this optional coiling has the same consistency as the other obligatory types of coiling. For this purpose I have studied mitosis in the root-tips of various species of *Fritillaria*, and I am also indebted to Mr. G. Taylor

of the British Museum for material of *Nomocharis*, some species of which were once included in *Fritillaria*, and to Dr. Mather for material of *Crocus*.

2. Metaphase of Somatic Mitosis

The stage when the chromosomes are lying with their spindle attachment chromomeres, or *centromeres* as I propose to call them, on the equator of the mitotic spindle may be divided into three phases:—

(i) *Early Metaphase*: the centromere is apparently undivided, since the two chromatids are drawn close together on either side of this point. Elsewhere they are lying loosely associated and more or less coiled round one another. The ends that are lying outside the spindle are often turned away from one another and are probably moving freely in the cytoplasmic currents.

(ii) *Full Metaphase*: The centromere is still undivided. The chromatids are so closely associated throughout their length that the direction of their coiling is no longer identifiable.

Fig. 1. Metaphase of mitosis in a root tip of *Fritillaria pluriflora* ($2n = 24$). M chromosomes marked and the direction of coiling of their arms (L and R). $\times 2200$.

(iii) *Beginning of Anaphase*: the centromere divides so that the attachment or centric constriction first resembles a secondary constriction, the chromatids lying parallel on both sides of it instead of being drawn together (Fig. 6) and later becomes the middle of a loop as the chromatids are pulled apart. As this change proceeds the chromatids become more loosely held together throughout their length, going back to their early metaphase state, and the direction of coiling again becomes plain. The association does not become so loose that the coiling fails to obstruct the anaphase separation. In a pollen-grain mitosis the chromatids may be drawn into fine threads between the centromere and the place of association, presumably as a result of delay in uncoiling (Fig. 4).

Chromosomes in a nucleus usually synchronise in making these changes. Where they do not, it is found that a lack of orientation on the plate is correlated with lack of division of the centromere, as

Fig. 2. Early anaphase in *F. recurva* ($2n = 24 + f$). Centromeres, invisible, represented diagrammatically following Navashin's description at mitosis (1927) and the present writer's at meiosis. Fragment lies off plate with centromere undivided. m, M chromosomes. $\times 2200$.

Fig. 3. The same (triploid variety). Seven chromosomes at the beginning of anaphase, showing optional relational coiling. $\times 2200$.

Fig. 4. Side view of anaphase in a pollen grain of triploid *F. pudica*, $3x = 39$ ($n = 15$). Note tension produced by uncoiling in an M chromosome. $\times 2200$.

with univalents at meiosis (Figs. 2 & 6).

The relative importance of the first two stages varies greatly as between organisms. Thus in many plants with chromosomes of moderate size the second stage is rarely found and coiling can therefore be readily recognised at full metaphase. This may be illustrated by *Spiro-nema fragrans* (Darlington 1929, Fig. 3). Observations of the direction of coiling of the chromatids in this type are open to the objection that the relative freedom of the

Fig. 5. Metaphase in root-tip of *F. Elwesii*, showing relational coiling in M chromosomes and others ($2n = 24$). The 28 arms show 11 R, 11 L, 6 U. $\times 2200$.

Fig. 6. *F. Elwesii* showing relational coiling, opposite in different arms of M chromosomes. At left hand: top, late prophase; bottom, unoriented metaphase chromosome. At right hand: top, 3 chromosomes in polar view of metaphase; bottom, 5 chromosomes in side view of two stages of early anaphase. $\times 2200$.

association of the chromatids during the period of orientation on the spindle might allow external influences such as cytoplasmic streaming to remove old coils and institute new ones. I find indeed that the chromatid coiling shows no consistency at metaphase in *Spiro-nema*. The same is true of *Crocus graveolens*, and of *Pushkinia libanotica* ($2n = 14$, Fig. 7).

Fritillaria represents an opposite type in which the first stage is telescoped and the direction of coiling cannot be accurately recorded, as a rule, before the beginning of anaphase. Observations on this type, although more difficult, are likely to be of greater value since the relationship of the chromatids observed at the beginning of anaphase will not have been disturbed by external influences.

Fig. 7. Somatic mitosis in *Pushkinia libanotica* ($2n = 14$). $\times 4000$.

I will first consider relational coiling of the chromatids at metaphase of mitosis in the following species of *Fritillaria*: *F. Elwesii* Boiss., *F. verticillata* Willd. var. *Thunbergii*, *F. pluriflora* Torr., *F. recurva* Benth. ($2x$ and $3x$). All these species have ten pairs of chromosomes with sub-terminal centromeres (S) and two pairs with nearly median centromeres (M) and provisionally assumed to be homologous throughout the group. The M chromosomes are longer than the S, but the separate arms are shorter than the large arms of the S. The long arms of the chromosomes vary at random in the degree of coiling. Short arms and fragments never show coiling.

The two M pairs have the consistent property of one arm being L-coiled (with a left-hand screw) and the other R-coiled (with a right-hand screw). This applies to all the species mentioned, although these species vary in the relative size of the two arms, presumably as a result of structural change. One exceptional chromosome has been found in *F. Thunbergii*, where both arms had an L-screw (Fig. 8). In *F. Elwesii*, the longer arm of each of the M Chromosomes

is that with the L-screw; one of the M pairs having a shorter arm with a secondary construction always has an R-screw. The mitotic coiling is therefore consistent with that found in meiosis, in which the arms are coiled in opposite directions, although whether the R-coiling at mitosis corresponds with R or L at meiosis cannot be said.

Fig. 8. Early anaphase M chromosomes in *F. Thunbergii* ($2n = 24$) showing opposite coiling in two arms except in one case. First three from one mitosis. $\times 2200$.

In *Fritillaria ruthenica* Wikstr. there are five pairs of M chromosomes, some with both arms having an R-screw, others with both arms having an L-screw. (Recorded: 11 L/R; 5 L/L; 2 R/R). The extra M chromosomes in this species are, I presume, derived (by unequal interchange), from pairs of S chromosomes in the other species. In *F. ruthenica* I also found indications of a coiling relationship that

Fig. 9. Metaphase and anaphase M chromosomes in *F. ruthenica* ($2n = 18$) showing optical relational coiling. First a polar view of metaphase. U, uncoiled. $\times 2200$.

seems to be analogous to what I have described as "redistribution" between the coiling of chromosomes in the two arms of the M chromosome bivalents in *F. Elwesii* (cf. also Koller 1935). The coiling direction extends from one arm into the other and changes in the middle of this arm (Fig. 8, arms marked).

Fig. 10. Mitosis in root tip in *Nomocharis saluenensis* ($2n = 24$). The 28 arms show: 9 R, 14 L, 5 U. $\times 2200$.

The related species *Nomocharis saluenensis*¹⁾ I. B. Balfour has a complement of the normal *Fritillaria* type but with smaller chromosomes. Their behaviour is more like that of *Spironema* and inconsistencies arise at metaphase in the direction of coiling of particular arms (Fig. 9, L). The coiling of M chromosomes so far as

it is consistent within the arm is constant as between chromosomes however.

3. Conclusion

The relational coiling of the chromatids seen in early prophase must result from the division of the chromosomes at the end of the resting stage along a cleavage surface that will enable the chromatids to lie parallel at metaphase and separate freely at anaphase. This original relational coiling must therefore disappear along with the relic coiling during prophase, and the behaviour of ring chromosomes in *Crepis* (Navashin 1931) confirms this view. The coiling at metaphase must depend, like all coiling between free threads, on the specific lateral attraction between them. It must be due to the chromatids being dragged round one another during spirallisation, and since it is optional the force or forces determining it must be variable in relation to the lateral attraction. I should expect these forces to depend on the assumption of the new spiral by each chromatid and therefore to be as consistent and characteristic in direction as the molecular spiral of the chromosome.

1) I am indebted to Mr. Taylor for the following species: *N. saluenensis*, Ibb; $2n = 24$. *N. Mairei*, Leveille; $2n = 24$. *N. pardanthina*, Franch.; $2n = 24$. *N. nana*, $2n = 48$ ($= 4x$).

This view is supported by the observed properties of the ring chromosomes; their structure is an insuperable obstacle to consistent relational coiling. If this coiling is an effect of spiralisisation, representing a wastage of the energy applicable to spiralisisation, then the ring chromosomes might carry their spiralisisation further than free chromosomes. This they frequently do, as Navashin's illustrations show.

The expected consistency seems to hold for the M chromosomes in *Fritillaria*, in which the direction of these spirals is consistent with that of the relational coiling of chromosomes I found at meiosis (1935 b). The exceptions including those dealt with elsewhere (Darlington 1936a) are important, but it is not worth while discussing them until fuller data are available.

4. Summary

1. The daughter chromatids are optionally coiled round one another at the metaphase of mitosis. The direction of coiling is usually consistent in each arm of all chromosomes in *Fritillaria* and constant for the identifiable M chromosomes. In *Nomocharis* however the direction is sometimes inconsistent in particular arms.
2. Chromatid coiling at metaphase seems to be developed during prophase chiefly as a result of a strain imposed on the chromatids by spiralisisation but subject also to other conditions that have not yet been ascertained.

5. Bibliography

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