The Hydration and Dehydration Phenomena in Mitosis

IV. The chromonemata as natural existence¹

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Received July 24, 1941

Some authors, especially those who consider the structure of the nucleus and chromosomes from the colloid-chemical point of view, have doubted that the chromonemata are of natural existence. SCHAEDE (1929) says: "Unter der Voraussetzung gallertartiger Beschaffenheit werden wir allen Strukturen, die in Kernfaden und Chromosomen beschrieben worden sind, mit Misstrauen begegnen müssen." The same author (1935) has later indicated a structure very much reminding us of the spiral structure of chromosomes, which he obtained from egg white fixed and stained as the chromosomes usually are. While such is the situation of the question at issue on one hand, there are, on the other hand, evidences which indicate that the chromonemata must be the structure of natural existence, evidences such as follows:—

1. In the living state of cells, it is revealed under the microscope that the chromonemata (or speaking in terms of the chromosome cycle the chromatids) in the chromosomes in telophase directly form the nuclear threads in the resting nucleus (KUWADA and NAKAMURA, 1940). Putting it in other words, we may say that the nuclear threads are the chromonemata.

2. SINKE (1937, 1939) has demonstrated that in the living nucleus in the resting stage the chromonemata which are visible in the isotonic solution are rendered invisible if the cells are treated with a hypertonic solution, and that when these dehydrated cells are hydrated by means of a hypotonic solution the chromonemata become visible again. SINKE has also found that this phenomenon of the chromonemata being rendered visible or not visible according to whether the cell is hydrated or dehydrated is taking place in the natural state in the guard cell nuclei of stomata when they open or close. In the living state of the cell the metaphase and anaphase chromosomes show no clear chromonema structure within, but the spiral structure becomes visible in the chromosomes when the chromosomes previously hydrated are dehydrated to the appropriate degree, and not visible again when the dehydration goes

¹ The cost of this investigation has been defrayed from the Scientific Research Expenditure of the Department of Education.

further (*Trillium* pollen mother cells, SINKE, 1939). It is, therefore, because the chromosomes are in so dehydrated a condition in metaphase and anaphase, that they show no chromonema structure in the intact state.

3. In the normal telophase, the chromonemata become visible, because the chromosomes are hydrated in this stage as a natural process¹. That the nucleus is hydrated in the telophase is evident from the fact that it grows considerably in volume during this stage from its contracted form in tassement polaire, a contraction stage which is observed in the living as well as in the fixed state. In this case of telophase the chromosomes become finally lost their outlines, probably owing to a strong hydration of the chromosome matrix, and the nucleus presents a diffuse structure. An artificial hydration of the chromosome matrix, too, can bring the chromosomes in metaphase and anaphase into the same state as that in the resting stage due to the telophasic transformation (cf. especially SIGENAGA² among others). It is demonstrated by KUWADA and NAKAMURA (1934a) with an artificial chromosome dissolution method that these products of nuclear structure from the metaphase and anaphase chromosomes are not those due to such an internal differentiation or rearrangement in the chromosomes as was once supposed by some earlier workers as taking place as a natural process in the chromosome cycle. While in the case of living material there is some interval of time before the chromosome assumes the structure like the nucleus, in the case of the dissolution method being used the chromosomes are instantly killed by the fluid, and hence there is, in the latter case, no room for thinking that differentiation or rearrangement to produce chromonemata occurs in the chromosome. The chromonema structure must exist already in the chromosomes in both metaphase and anaphase.

From these results of observation and experiments, we may say that the chromonemata exist in all the stages in the chromosome cycle, if the prophase is excluded. In the present paper we want to emphasize that in our recent publications it is shown that the actual existence of the chromonemata is demonstrable in the prophase also.

It has already been shown that the chromonema spirals are visible in the natural state in the spiral stage of prophase (KUWADA and NAKAMURA, 1934b), but the identification in the living state of the spiral threads to the chromonemata in the resting nucleus has remained undetermined. This identification of the two structures can be attained only by the investigation of the transition stage from the resting to the mitosis or the stage in the very beginning of mitosis. Having this in

¹ In the telophase chemical changes in quality or quantity should take place, and in this case these changes might have some connection in making the chromosome hydration take place so much easier that it occurs regularly in the telophase of the normal mitosis.

² Preliminary report in 1937.

view, we watched resting nuclei of staminate hair cells of *Tradescantia reflexa* under the microscope. In several cases the nucleus began the mitosis as we expected. Two of these cases have been recorded in another paper (KUWADA and NAKAMURA, 1941). In the present paper two other cases will be mentioned.

Case 1. The observation was begun at 2.26 p.m. (Fig. 1). The nucleus showed some clear aspects of aggregate structure. At 2.29 the structure appeared more or less diffuse. At 2.48 and 3.00, the nucleus showed again an aggregate structure, and at 3.37 the diffuse structure. At 4.28, clear interchromosomal spaces were visible—the *b* stage¹, and then the nucleus slightly increased in volume and the interchromosomal spaces became more distinct. In the interval from 4.31 to 4.41, the nucleus presented the typical structure in the spiral stage. The changes in the *a* stage¹, i.e. the stage before the *b*, in apparent structure of the nucleus, sometimes showing an aggregate structure, sometimes a diffuse structure, were also observed in the two other cases given in the other paper. These changes may be connected with the changes in water relation in the nucleus. In all these cases, however, city water was used as medium, and it remains undetermined whether the changes are natural phenomena or phenomena due to the condition of the medium being water. Nevertheless, it seems that the *a* stage is a preparatory stage for the mitosis, and that in this stage a change toward a more regular arrangement of the chromosomes is taking place in the nucleus, probably in some relation to the polar activity of the cell.

Before 3.37, most chromatids appeared to show some twisted configuration, and at 4.28 was first observable a clear spiral configuration characteristic of the nucleus in the spiral stage. It seems likely that the nucleus becomes hydrated when it is entering the spiral stage, probably preceded by a dehydration as suggested by the dehydration-hydration experiment by SINKE (KUWADA, SINKE and NAKAZAWA, 1939, Fig. 1*d*) on the resting nucleus, and that as a result of the hydration the twisted configuration of the chromatids (chromonemata) in the resting nucleus is transformed into the spiral configuration. This transformation of the configuration is more clearly demonstrated in the following case.

Case 2. The observation was begun at 2.45 p.m. (Fig. 2). The nucleus showed the coarse structure with chromatids of twisted configuration or of the dotted structure². At 2.50, i.e. 5 minutes later, the "dots" became swollen, and at 3.03 the spiral configuration was observed more or less clearly (comp. KUWADA and NAKAMURA, 1940, Fig. 7*a, b*).

¹ KUWADA and NAKAMURA, 1941.

² In this case 2, a 5% alcohol was used as medium, and accordingly a doubt arises as to whether this coarse structure of the nucleus is not an effect of the alcohol used. We have, however, observed this type of coarse structure in the water medium also (KUWADA and NAKAMURA, 1940). The question is left for further investigation.

Fig. 1. A staminate hair cell of *Tradescantia reflexa* mounted with city water at 2.20 p.m. *a.* At 2.26. *b.* At 2.29. *c.* At 2.48. *d.* At 3.00. *e.* At 3.37. *f.* At 4.28. *g.* At 4.31. *h.* At 4.36. *a-e.* Stage *a.* *f.* Stage *b.* *g-h.* Stage *c* (spiral stage).

At 3.17 and 3.32 the nucleus was in the typical spiral stage, the spiral configuration now being very distinct. In this case the nucleus began to turn back to the resting stage at 3.55 (a prophasic restitution).

Fig. 2. A staminate hair cell of *Tradescantia reflexa* mounted in 5% alcohol at 2.43 p.m. a. At 2.45, 2 minutes after mounted. b. At 2.50. c. At 3.03. d. At 3.17. e. At 3.32. In this case the nuclear threads in a (stage a) are of the typical twisted configuration, which is rather rare, so far as our investigation is concerned (comp. Fig. 7a in the previous paper, 1940). b. Stage b. c. Later stage. d-e. Stage c.

All these cases are the results of the observation of living material, made during mitosis. The nuclear threads or chromonemata are thus

From the results of the observation mentioned above we see that the nuclear threads in the resting stage directly form the spiral threads in the spiral stage—a demonstration of the chromonemata as actual existence in the early prophase chromosomes.

The spiral threads or coiled chromonemata become visible in the chromosomes in the mid-prophase too, if the chromosomes are hydrated as an abnormal phenomenon, and they may form directly the thread structure of the nucleus through the process of restitution. Some of these cases have been described and illustrated in the other paper (KUWADA and NAKAMURA, 1941). In the same paper it is moreover shown that the chromosomes can be transformed in every stage of prophase into the structure of the resting nucleus through the same process.

demonstrated as existing in the prophase too. We may now conclude that they must exist throughout the chromosome cycle. They can be no product of artifact.

SINKE (1937, 1939) has demonstrated experimentally that when a nucleus is dehydrated or hydrated to a high degree the nuclear structure disappears from sight, but comes to sight again when this nucleus is hydrated or dehydrated reversely. In these cases the change in refractivity is responsible for the visibility or non-visibility. Non-visibility implies in no sense that the chromonemata do not exist. They exist really throughout the chromosome cycle, but when they are not visible, this is only owing to some definite condition, mainly one that causes hydration or dehydration. There is also no reason to suppose, as indicated above, that the chromosome spirals are products of the telophasic differentiation in the chromosomes (BONNEVIE, 1908) or by rearrangement in the prophase (VEJDOVSKÝ, 1911–1912). The spiral threads or the chromonemata are produced only by division from the pre-existing chromonemata (KAUFMANN, 1926 and others) as are the chromosomes from the chromosomes, the nuclei from the nuclei, the cells from the cells, all by division. They are not brought forth by differentiation nor by rearrangement in the chromosome, but undergo respiralization, which may mislead one to the conception of the new formation, in the early prophase of each mitosis as clearly demonstrated by AISIMA (1941) with his maceration method. The older view that the chromosome spiral or the chromonema is a product of differentiation or rearrangement in each mitosis is to be replaced by the statement that it is reproduced by division in each chromosome cycle, and by this replacement the weak point of the chromonema theory which WILSON (1925) pointed out can be removed.

Summary

1. It is contended that the chromonema must be a natural structure.
2. The results of observations presented in the present paper are as follows:—

- a) The chromonemata are visible in living chromosomes in prophase, in the spiral stage in the natural state and in the mid-prophase in a hydrated state.

- b) In the very beginning of mitosis the nucleus is hydrated probably preceded by a dehydration of the nuclear elements. As a result of these dehydration and hydration the interchromosomal spaces first become visible.

- c) Then the twisted configuration or “dotted structure” of the nuclear threads in the preparatory stage of mitosis is transformed into the spiral configuration, thus the spiral stage being reached. The trans-

formation begins with the swelling of "dots" indicating that in this outward aspect the spiral turns of the tightly twisted form are loosened gradually by hydration to reassume the regular spiral form.

Literature Cited

- Aisima, T. (1941), Studies of mitosis and meiosis in comparison. II. Chromosome structure in the spiral stage and anaphase in mitosis as revealed by means of a maceration method. *Cytologia*, 11.
- Bonnevie, K. (1908), Chromosomenstudien. *Arch. f. Zellf.*, 1.
- Kaufmann, B. P. (1926), Chromosome structure and its relation to the chromosome cycle. I. Somatic mitoses in *Tradescantia pilosa*. *Amer. Journ. Bot.*, 13.
- (1926), Chromosome structure and its relation to the chromosome cycle. II. *Podophyllum peltatum*. *Amer. Journ. Bot.*, 13.
- Kuwada, Y. and Nakamura, T. (1934a), Behaviour of chromonemata in mitosis. II. Artificial unravelling of coiled chromonemata. *Cytologia*, 5.
- and — (1934b), Behaviour of chromonemata in mitosis. III. Observations on living staminate hair cells in *Tradescantia reflexa*. *Mem. Coll. Sci. Kyoto Imp. Univ. S. B.*, 9.
- and — (1940), Behaviour of chromonemata in mitosis. IX. On the configurations assumed by the spiralized chromonemata. *Cytologia*, 10.
- and — (1941), Studies of mitosis and meiosis in comparison. V. Some experimental observations of mitosis in *Tradescantia* staminate hairs. *Cytologia*, 12.
- , Sinke, N. and Nakazawa, Z. (1939), The hydration and dehydration phenomena in mitosis. II. A consideration of the spiral stage with the results of experiments and observation. *Cytologia*, 9.
- Schaede, R. (1929), Die Kolloidchemie des pflanzlichen Zellkerns in der Ruhe und in der Teilung. *Ergeb. d. Biol.*, 5.
- (1935), Beiträge zum Artefaktproblem. *Protoplasma*, 23.
- Sigenaga, M. (1937), An experimental study of the abnormal nuclear and cell divisions in living cells. *Cytologia*, Fujii Jub. Vol.
- Sinke, N. (1937), An experimental study on structure of living nuclei in the resting stage. *Cytologia*, Fujii Jub. Vol.
- (1939), Experimental studies of cell-nuclei. *Mem. Coll. Sci. Kyoto Imp. Univ. S. B.*, 15.
- Vejdovský, F. (1911–1912), Zum Problem der Vererbungsträger. *Prag*.
- Wilson, E. B. (1925), The cell in development and heredity. 3rd ed. New York.
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