THE RÔLE OF THE "CHROMOSOME SHEATH" IN MITOSIS, AND ITS POSSIBLE RELATION TO PHENOMENA OF MUTATION

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Until recently, cytologists who have considered chromosome movements and chromosome associations have given little attention to the possible rôle of the "chromosome sheath" or envelope—the transparent and apparently gelatinous layer of material which may be seen under favorable conditions surrounding the stained chromosome. This neglect has been due, presumably, to the fact that in most cases such a structure is not visible and that in consequence there is no certainty that the "sheath" is characteristic of chromosomes in general. There seem to be good grounds, however, for considering that the sheath is such a typical structure, and also for considering that it may play an important rôle in the activities of the chromosomes, particularly during mitosis. Such consideration, furthermore, when taken in connection with recent evidence concerning the artificial induction of mutations, suggests that the chromosome sheath may likewise have a direct connection with the phenomena of mutation.

It is the purpose of the present paper to note very briefly some of the evidence on these points. Detailed treatment will be reserved for another paper.

As regards direct observation of the "sheath" there is, on the one hand, strong evidence that wherever conditions are suitable for revealing the presence of such a structure it is visible. The nature of this evidence has been noted in earlier papers[1] and need not be considered here. On the other hand, it is clear that commonly the sheath would not be visible because of the fact that the chromosomes lie in a medium (nucleoplasm or spindle substance) which, like the sheath, remains transparent even in stained preparations.

The evidence indicates that the sheath is a layer of differentiated material around the chromosome, fairly definitely delimited, but without any peripheral membrane or cortex. Presumably it is a product of the activity of the chromosome proper[2] and is characterized in part by its physical consistency. Perhaps it may even be continuous with, and similar to, the matrix of the chromosome proper. It appears to be present not only when the chromosomes are condensed, as during mitosis, but also when they are elongate and thread-like (Metz and Nonidez, loc. cit.).

Details as to the nature and appearance of the structure are not, however, of primary concern in the present connection. It is desired here to note particularly certain characteristics of chromosome behavior which indicate the presence of the sheath and the rôle which it may play even when it is not directly visible.

1. *The Rôle of the Sheath in Mitosis.*—Orientation of the chromosomes on the metaphase plate in mitosis, involving a definite spacing of the chromosomes, usually approximately equidistant from one another, is, in the writer's opinion, best accounted for on the assumption that the stainable part, or chromosome proper, is surrounded by a differentiated, gelatinous layer which keeps it from coming into contact with the other members. This feature has been elaborated somewhat in an earlier paper (Metz '28, loc. cit.).

Likewise, certain features of chromosome behavior, from prophase through early anaphase, which have never received adequate explanation, are subject to ready interpretation if the presence of a sheath is taken into consideration. It is well known that during late prophase and metaphase in many forms each chromosome is split along its entire length, with the two daughter halves distinctly separated from one another throughout. Yet these daughter halves may remain accurately aligned and approximately equidistant from one another, even in the case of long chromosomes and during a period of time in which the chromosomes undergo considerable movement.

The persistence of this accurate alignment has been attributed to an hypothetical "attraction" between the daughter halves, although it has been recognized that in other respects chromosome behavior during this period indicates a repulsion rather than attraction between the daughter halves. It is clear that the original division or splitting of the chromosome is an autonomous act, and that some sort of repulsion serves to effect a slight separation of the daughter halves. Their failure subsequently to fall apart, or become more widely separated, during prophase and metaphase movements, and also the relative absence of twisting and other irregularities of alignment are not only understandable on the assumption of an enveloping sheath, but serve in themselves to suggest the presence of such a structure.

Even more striking evidence is provided by the subsequent behavior of the elements in question. It is seen most clearly, perhaps, in the case of long chromosomes which at metaphase extend some distance across the equator of the spindle or even out of it. In some organisms, as the poleward movement of the daughter halves of such chromosomes progresses, beginning at the "spindle fibre" locus, the point of divergence is sharply delimited and each half is bent almost at right angles at this point, the unseparated arms remaining in close association up to the point of

divergence. In such cases the point of divergence between the two halves may remain approximately fixed within the spindle while the arms move in toward it as separation progresses.

Such behavior receives a simple explanation if it is assumed that the enveloping sheath serves at first to hold the two sister chromosomes together and that it gradually undergoes longitudinal division as separation progresses, beginning at the spindle fibre locus. Variations in the degree of association and in the behavior of the sister halves in different organisms would, on this view, depend on variations in the time and rate of division of the sheath. The same principle may be applied to homologues in meiosis.

Evidence has been presented in another paper[3] which suggests that the chromosome sheath may take an active part in causing the movement of the chromosome toward the pole in anaphase, after separation of the daughter halves has taken place, and that to this extent the movement of the chromosome may be autonomous. A somewhat similar suggestion has been made in earlier papers by Bleier (loc. cit.) but on his view, as noted above, the material enveloping the chromosome is independent of the chromosome itself in both origin and activity.

If the above considerations are valid it would appear that the chromosome sheath plays an important rôle throughout the whole process of mitosis (including meiotic divisions).

2. *Possible Relation of the Sheath to Mutation.*—On the assumption that the sheath is a characteristic structural component of chromosomes it seems probable from the available evidence that one of its primary functions is that of insulating the chromosome proper and preventing it from coming into direct contact with other formed bodies in the cell, including the other chromosomes.[4] Indications of this are seen not only in the spacing of the chromosomes on the equatorial plate, as noted above, but in their relations at all other stages. It would appear, therefore, that anything tending to disperse or otherwise alter the chromosome sheath might readily lessen or eliminate its insulating properties and permit the chromosomes to come into direct contact with one another. This consideration suggests a possible important relation of the sheath to mutation phenomena.

The well-known studies of Muller, and subsequently of many others, on the artificial induction of mutations, have shown clearly that irradiation with x-rays or radium greatly increases the rate of mutation in numerous organisms. Likewise, it greatly increases the frequency of occurrence of breaks in chromosomes, and of translocations, inversions and "reduplications" of parts of chromosomes. By comparing the incidence of gene mutations with that of the gross changes (translocations, etc.) Muller has secured evidence leading to the view[5] that the underlying process

which results in the former is essentially the same as, or bears a significant resemblance to, that which results in the latter type of change. Furthermore, since the gross changes are evidently not the result of direct modification of the molecular structure, it is inferred, on this view, that gene mutations may not be the result of such direct modification, but may be due to intimate contact and interaction between chromosomes such as does not occur frequently under ordinary conditions. "The irradiation has somehow (possibly by de-charging them) done away with the repulsion which normally holds chromosome strands apart from one another. . ." (Muller, loc. cit., p. 218).

Taken in connection with the considerations presented above, these findings suggest immediately that irradiation serves to disturb the normal insulating properties of the chromosome sheaths and permits intimate contacts between the chromosomes. It seems possible that such contacts may be a primary cause of mutation, particularly when they occur at a stage in which the chromosomes are long and thread-like. Such an interpretation would apply similarly to the effects of high temperature, which has also been shown to stimulate mutation.[6]

The interpretation just outlined is in harmony with the observed effects of irradiation and heat on chromosomes. Several investigators have shown[7] that these agents cause a clumping of chromosomes, often accompanied by fusion and subsequent fragmentation of some members. The observations indicate, at least in the case of irradiation with x-rays or radium, that a decrease in protoplasmic viscosity is produced.[8] On the present view this serves to solate the gelatinous "sheath" permitting chromosomes to come into contact with one another and to interact in such a way as to cause segmental rearrangements and gene mutations.

If this interpretation is correct it follows that any agent reducing the insulating properties of the chromosome sheath in this manner might increase the rate of mutation. Muller (loc. cit.) has suggested that if the cause of gene mutations is essentially the same as that of translocations, etc., it might be expected that other agents, including chemicals, would be effective in increasing the mutation rate. The present observations tend to support such an inference and to suggest the mechanism which may underlie the phenomena.

[1] Metz and Nonidez, *Biol. Bull.*, **46**, 153 (1924); Metz, C. W., *Genetics*, **10**, 345 (1925); Bleier, H., *La Cellule*, **40**, 82 (1930); *Genetica*, **13**, 27 (1931); Koerperich, J., *La Cellule*, **39**, 309 (1930); Schrader, F., *Zeits. f. wiss. Zool.*, **142**, 520 (1932).

[2] Bleier (loc. cit.) considers it as having an independent existence, but the evidence for this view does not appear convincing. See also Schrader, loc. cit.

[3] Metz, C. W., *Biol. Bull.*, **64**, 333 (1933).

[4] It does not, however, act merely as an inert layer. It must undergo numerous changes and it apparently has the power of selective action, as shown, e.g., by the fact that it does not prevent synapsis of homologous chromosomes.

[5] Muller, H. J., *Proc. VI Int. Cong. Genet.*, **1**, 213 (1932).

[6] See, e.g., Goldschmidt, R., *Biol. Zbl.*, **49**, 437 (1929); Jollos, V., *Ibid.*, **50**, 541 (1930), *Naturwis.*, **19**, 171 (1931), *Ibid.*, **21**, 455 (1933) and **21**, 831 (1933); Rokitzky, P. T., *Biol. Zbl.*, **50**, 554 (1930); Plough and Ives, *Proc. VI Int. Cong. Genet.*, **2**, 156 (1932); Grossman and Smith, *Amer. Nat.*, **67**, 429 (1933).

[7] See, e.g., Koernicke, M., *Ber. d. Deuts. Bot. Gesel.*, **23**, 404 (1905); Takimine, N., *Bot. Mag. Tokyo*, **37**, 109 (1923); Packard, C., *Quart. Rev. Biol.*, **6**, 253 (1931); Helwig, E. R., *J. Morph.*, **55**, 265 (1933); Lewis, M. R., *Arch. f. exp. Zellf.*, **14**, 464 (1933).

[8] Williams, M., *Ann. Bot.*, **39**, 547 (1925); Helwig, loc. cit. (1933).

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*DEVELOPMENT OF THE EMBRYO SAC OF LILIUM HENRYI**

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While making a study of cell-plate formation in the macrogametophyte of *Lilium Henryi* Baker, the writer observed that the type of embryo-sac development was at variance with the so-called "lily type." Instead of the egg being removed from the macrospore mother cell by three divisions, four divisions actually intervene.

The nucleus of the macrospore mother cell and its daughter nuclei pass through the heterotypic and homoeotypic divisions without cell division so that a four-nucleate embryo sac is formed. A polar view of the homoeotypic equatorial plates shows 12 chromosomes on each spindle (Fig. 1). Three of the four nuclei formed as the result of the homoeotypic division pass to the chalazal end of the embryo sac (Fig. 2), and then all four nuclei divide simultaneously. During this division the spindles of the three chalazal nuclei unite. A multipolar spindle is evident at relatively early stages (Fig. 3), but ultimately a single bipolar spindle is formed and the 36 chromosomes becomes arranged on a common equatorial plate. In figure 4 both equatorial plates are visible in polar view. Twelve chromosomes can be counted on the spindle at the micropylar end of the embryo sac, whereas there are 36 on the chalazal spindle.

As a result of this division a four-nucleate embryo sac is again formed. Two small nuclei are near its micropylar end and two larger nuclei at the chalazal end. Earlier investigators (Sargant,[1] Mottier[2]) explained the greater size of the two chalazal nuclei as probably due to growth because this condition was found only in the larger embryo sacs. The spindles of the third division just referred to persist between the perinuclear zones which are formed about each daughter nucleus. The embryo sac elongates to some extent before the nuclei divide again.

Three of the four nuclei now present, the two at the micropylar end of the