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On the Mitotic Movements of Chromosomes

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It is my purpose to bring together some of the published and unpublished results that my coworkers, students, and I have obtained in recent years in studying mitosis and the effects of certain physical, chemical, and mechanical treatments on it. Anyone who has watched mitosis in the living cell cannot fail to have asked himself what causes the regular, precise succession of nuclear and cytosomic changes associated with division. Ever since the main morphological features of mitosis were observed in the 1870's, cytologists and cellular physiologists have speculated about the nature of the forces responsible for the various phases of chromosome orientation and movement in the dividing cell. The universality of mitosis in the plant and animal kingdoms, its preciseness in distributing through countless cell generations exact halves of each chromosome to each of the many kinds of cells that comprise the organism, and the amazing similarity in the process even in the most diverse kinds of cells suggest that, in an evolutionary sense, very basic phenomena are involved. A mass nuclear division called amitosis is known to occur in some organisms, but it is certainly rare. Mitosis is, indeed, almost as universal a phenomenon of nuclear reproduction as the cell is a unit of organismal structure.

A critical review by Schrader of the literature on the movements of the chromosomes in cell division has appeared recently (*1*); therefore, I have limited my remarks to certain aspects of this problem on which our recent studies of grasshopper neuroblasts have a bearing. Neuroblasts are large cells that exist temporarily on either side of the midventral line of the embryo early in development. They occur on or close to the surface and lay down by successive divisions smaller ganglion cells that differentiate to form the cells of the ventral nerve cord. Their large size and location at the surface make it possible to observe mitosis in great detail in hanging-drop culture preparations. The results we have obtained with the neuroblast probably hold for many, but not all, kinds of cells. The grasshopper we have used is *Chortophaga viridifasciata* (De Geer).

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Telophase-Interphase-Prophase Orientation

A striking feature of many cells, and in particular of the grasshopper neuroblasts, is the close resemblance between the chromosome orientation at telophase (Fig. 1) and at the succeeding late prophase (Fig. 2). In each, the spindle attachment ends, or centromeres, of the chromosomes are clustered in a group close to or against the nuclear membrane, and the distal ends radiate outward and toward the sister cell. In the intervening stages, however, the long, twisted chromatin threads appear to have no orderly arrangement within the nucleus. One means of accounting for similarly oriented chromosomes separated by a stage of apparent disorder is to assume that the chromosomes are not free within the nucleus, but are attached in a limited region of the nucleus from the time it arises in telophase until it disappears at the end of prophase. The logical points of attachment would be the centromeres of the chromosomes. There is experimental evidence from microdissection, radiation, and colchicine studies that the chromosome centromeres have a regular, fixed orientation within the nucleus.

If a microneedle is inserted in the nucleus of a neuroblast in late prophase and moved crosswise to the chromosomes, their distal ends can be displaced more readily than their proximal ends, which behave as if they are fastened in a group at or near the nuclear membrane.

Certain kinds of chromosome translocations induced in interphase or prophase by ionizing radiations are manifest in the succeeding metaphase as chromosomal elements with two centromeres each, known as dicentrics. It happens rarely, if ever, that these two centromeres are adjacent to each other on the metaphase spindle of the neuroblast. Nearly always, one or more chromosomes intervene, and these together with the including dicentric are often crowded quite closely together as compared with the others, which are more widely spaced than usual on the periphery of the spindle. This suggests that the position of the centromeres with respect to each other is fixed by attachment to some part of the nucleus or the inner surface of the nuclear membrane early in prophase at the time when the translocation was induced and before the late-prophase shortening of the chromosome has occurred, and that this attachment is sufficiently strong to be maintained into metaphase, even at the expense of crowding the intervening centromeres.

X-ray-induced chromosome breakage in *Tradescantia* microspores has been found by Sax and Mather (*2*) to occur with greater frequency at the centromeric than at the distal ends of the chromosomes. The breakage frequency was also greater in chromosomes with centromeres than in acentric fragments, which lack centromeres (*3*). These effects are interpreted as demonstrating greater torsional stresses near the centromere, which in turn implies that the maintenance of chromosome polarity during interphase and early prophase is due to some intranuclear attachment of the centromere.

Occasionally neuroblasts that are exposed to colchicine in late prophase are blocked at this stage for several hours.

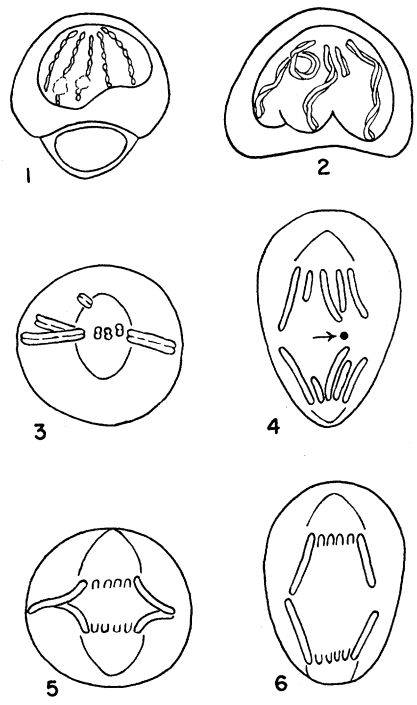
In such cells the chromosomes lose their telophaselike orientation inside the nucleus and come to lie at random with respect to one another (*4*). Since colchicine-induced destruction of the metaphase spindle results in a similar disorientation of chromosomes through loss of centromere attachments at a stage when chromosomes and spindle have replaced the nucleus, it is logical to suspect that the disorientation within the prophase nucleus has also resulted from destruction of a spindle or spindlelike material to which the centromeres are normally attached.

Metaphase Orientation

The metaphase arrangement of the chromosomes to form an equatorial plate raises certain interesting questions involving the relation of metaphase to earlier and later stages. If neuroblasts in very late prophase or prometaphase are treated with a relatively large dose of ultraviolet radiation of wavelength 2250 angstroms, normal development of the spindle is temporarily prevented (*5*). After the breakdown of the nuclear membrane, part of the clear material of the nucleus, instead of accumulating centrally to form a spindle, flows to one side of the cell to form a large clear globule. The remainder maintains contact with the chromosomes as an abnormally small and often irregularly shaped presumptive spindle, to which the chromosome centromeres are attached in a compact group, and from which the chromosomes extend outward in all directions. Often one or more of these chromosomes moves temporarily away from the others, but retains contact at its centromere with the spindle substance (Fig. 3). With the eventual organization of this material into a definitive but small spindle, such chromosomes slowly move toward and finally rejoin the main group, which, meanwhile, has formed an equatorial plate. By watching this movement, one can predict exactly when anaphase will begin, for it never starts until all the chromosomes are in the equatorial plate, and it always starts as soon as the last one has reached it.

M. E. Gaulden and I (*4*) have found a similar effect after prolonged treatment of neuroblasts with very low concentrations of colchicine, which, like short-wavelength ultraviolet, interferes with spindle formation. The colchicine concentration and duration of treatment are quite critical in the induction of this effect. In the neuroblast, interference is produced 3 to 4 hours after the cells are placed in a solution containing 1 part of colchicine to 25 million parts of culture medium. In such cells the metaphase spindle appears normal or almost normal, but one of the chromosomes may be attached to the spindle near the pole. It gradually moves toward the equator. Anaphase begins as soon as it has joined the other chromosomes in the equatorial plate. A similar effect resulting from heat treatment—specifically, exposure of neuroblasts to a temperature of 45° C for 6 minutes—has been observed by Gaulden 36 minutes after treatment.

More recently Bloom, Zirkle, and Uretz (*6*) have found a similar phenomenon in untreated tissue culture cells of an amphibian, the newt. They report that, after breakdown of the nuclear membrane, one of the chromosomes in about half the cells is attached near the pole of the spindle at a time when the others are in the equatorial plane. After an interval that may amount to an hour or more, this chromosome moves to the equator. Once it has lined up with the other chromosomes, anaphase separation of the chromosome halves begins.



Figs. 1–6. Selected mitotic stages of the grasshopper neuroblast, redrawn from camera lucida sketches of living neuroblasts *in vitro*. Only a few of the chromosomes actually present are shown, these having been selected to illustrate chromosome position and orientation. *1,* Late telophase; *2,* late prophase; *3,* metaphase, in which one of the chromosomes is separated from the rest and the spindle is reduced in size after treatment with 2250-angstrom ultraviolet radiation; *4,* middle anaphase, demonstrating presence of invisible interzonal fiber (the black dot and arrow represent the microneedle and the direction of movement of the microneedle, respectively); *5,* early anaphase, illustrating stage when elongation of the spindle begins; *6,* middle anaphase, illustrating stage when elongation of the spindle ends.

I am inclined to believe that in these four examples we have the same situation, even though three were of treated and one of untreated cells. The feature that they have in common is an incomplete spindle. Incompleteness is due in the first three examples to retardation of spindle development by ultraviolet radiation, colchicine, or heat, and in the fourth to normal immaturity of the spindle. This chromosomal phenomenon raises three interesting questions.

First, why do most of the chromosomes retain their close proximity to one another with usually only one—or occasionally two or three—becoming separated from this main group? In my opinion we have to deal with two opposing forces when we attempt to analyze the prometaphase-metaphase movements and orientation of the chromosomes. One force tends to keep the centromeres of the chromosomes at the equator of the spindle and incidentally, therefore, in close proximity to one another. We may assume that this force involves an interaction of spindle and centromere. The other force is manifest as a repulsion between chromosomes. As a result of this force, the distal portions of the metaphase chromosomes radiate outward from the spindle like the spokes of a wheel, and there is a tendency in the grasshopper neuroblast for adjacent chromosomes to lie on opposite sides of the spindle equator (*7*).

It may be that in the partially destroyed or incompletely formed spindle the spindle-centromere force is reduced to the extent that the interchromosomal repulsion force is great enough to overcome it and move one or more chromosomes from the equator out along the spindle. Return of the separated chromosomes would then occur with restoration or development of the spindle to the point where the spindle-centromere force tending to move the chromosomes toward the equator is greater than the interchromosomal repulsion forces tending to keep them apart.

Second, why does the anaphase separation of chromatids begin only after all the chromosomes in these cells are in the spindle equator? All I can suggest is that perhaps the force that moves sister chromatids toward opposite poles of the spindle is not divisible functionally into a series of individual forces acting on the chromosomes independently but must act as a single unit in separating all the sister chromatids. This idea is supported by the nature of the physical changes that take place in the spindle during anaphase. Movement of the centromere groups to the poles is accom-

panied by liquefaction of the spindle material between them. It is difficult to interpret this as a series of liquefactions involving separately the different pairs of sister chromatids.

Third, why does the anaphase separation of chromatids begin as soon as the chromosomes in these cells are all in the spindle equator? The chromosomes of nearly all cells about which we have information normally occupy the metaphase position in the cell for at least a few minutes before anaphase separation of chromatids begins. In grasshopper neuroblasts at 38° C, metaphase lasts about 9 minutes. Therefore, something in addition to the metaphase orientation initiates anaphase. During this time the doubleness of the chromosomes becomes more pronounced, so that after a short time sister chromatids, though still connected along their whole lengths, are clearly distinguishable. This occurs at about the same rate whether or not the spindle has been altered by experimental agents. Probably there is some progressive change in the spindle that must attain a certain state in order to initiate anaphase. In cells in which the spindle is damaged by experimental agents or in which the forces acting between the spindle and chromosomes have not developed to the point where all the chromosomes have been moved into the equator, we might suppose that intrinsic chromosomal forces adequate for chromatid separation await only a certain condition of the spindle. As soon as the spindle has recovered or developed to the point where the last chromosome reaches the metaphase plate, anaphase separation is triggered off.

Anaphase Movement

If we are to consider intelligently the part the spindle plays in anaphase, we must first reach a common understanding of the physical nature of the living spindle and the changes it undergoes during anaphase. The metaphase spindle of the grasshopper neuroblast is a semisolid structure, which, together with the attached chromosomes, can be pushed about with a microneedle in the more fluid, surrounding cytoplasm (*8*). It maintains its shape as it is moved, unless it is pressed against the cell membrane. Then it is temporarily deformed, but on release of the pressure it gradually resumes its original shape. It is rigid enough to resist penetration by any but a very sharp needle, rolling to one side as the tip of a blunt needle is pressed against it. Spindle fibers are not visible, but there is good evidence of a longitudinal orientation of spindle components. If a microneedle is inserted into the spindle, it can be moved slowly toward the end of the spindle, eventually passing to the outside, but it cannot be moved through the spindle crosswise. Anaphase is marked by liquefaction and, during a limited period, elongation of the spindle. The evidence of elongation of the spindle is a gradual increase in the interpolar distance. Elongation begins soon after the proximal ends of the chromatids have started to separate (Fig. 5) and ends when the distal ends have mostly lost contact with one another (Fig. 6). Liquefaction begins at the equator as the chromosomes start to separate and extends progressively toward the poles with or slightly in advance of the separating centromeres. By middle anaphase all the original spindle is highly fluid except the small part between the centromeres and the poles, and this region has decreased in viscosity to such an extent that the microneedle can be moved through it with the displacement of only those chromosomes in its path. Mitochondria have moved into this region, where they show rapid Brownian movement. The only other nonfluid structures that can be demonstrated in this region are invisible strands known as interzonal fibers that are presumably derived from the outer nonstaining covering of the chromosomes and that connect the distal ends of the sister chromosomes. They can be demonstrated by moving the microneedle crosswise in this region, which causes a movement of the joined sister chromosomes in a similar direction (Fig. 4). The strength of these fibers is demonstrated by the fact that they can be stretched to several times their normal length without breaking (*8*).

Among the hypotheses that have been proposed to explain anaphase separation of chromatids are spindle fiber contraction, magnetic forces, electromagnetic forces, protoplasmic currents, interzonal region elongation, autonomous movement of chromatids, and various combinations of these. It is not my present intent to evaluate extensively the evidence for or against these various hypotheses, but rather to discuss some of my own experiments and observations that bear on this problem.

Anaphase separation of chromosome halves has often been assumed to depend on the division of their centromere. This is not true of grasshopper neuroblasts. (*9*). If these cells are exposed to x-rays, broken chromosomes in the form of proximal and distal fragments are produced. The former contain centromeres; the latter lack them. At metaphase, the fragments with centromeres are attached to the spindle, while the acentric fragments lie in or near the equatorial plate at its periphery. During anaphase, the sister chromatids of these acentric fragments separate. Usually their separation begins at one end and proceeds to the other end. We may conclude, therefore, that neither the centromere nor a force acting through the centromere is essential in separating the sister chromatids from one another. Their separation is apparently autonomous. It is, however, somewhat different from that of the chromosomes attached to the spindle. The chromatids of acentric fragments remain relatively straight throughout separation, while the normal chromosomes are bent close to the spindle, at an angle approaching 90 degrees, as if there were an additional separating force involving the spindle and centromere. Their movement apart after separation, however, is another matter, for acentric fragments do not move toward the poles at anything approaching the rate of the chromosomes with centromeres. As a matter of fact, whether or not they end up in different daughter cells seems to depend on whether they happen to be pushed by the constricting late anaphase cell against the interzonal fibers at just the right place. If this occurs, the elongating interzonal fibers may move the sister chromatids far enough apart that they end up in separate daughter cells.

My microdissection studies of the neuroblast have convinced me that the attachment of the chromosomes to the spindle is so firm at all stages of anaphase that we can disregard the magnetic, electromagnetic, and protoplasmic streaming hypotheses as possible explanations of this movement. Anaphase separation may be assumed probably to depend, therefore, on a pulling action of a contracting spindle or a pushing action of the interzonal region between the two sets of separating chromosomes, or both. Let us consider first the various evidences for a pulling action of the spindle.

1) An early concept of spindle structure and one still held by many biologists is that the spindle consists of microscopically visible spindle fibers, certain of which are attached to the chromosomes. This is the picture one sees after use of certain fixatives and stains. The biologist associates fibers with contraction and sees in fixed preparations that the later the anaphase, as evidenced by the distance between the daughter chromosomes, the shorter the spindle "fibers."

2) The jerking motions that we see in "moving pictures" of mitosis and meiosis look to the casual observer like tugging or pulling motions. Such pictures are, in a sense, not true motion pictures, but rather time-lapse photography, in which a series of pictures taken at intervals of a few seconds apart are run off at a rate of 16 or 32 per second. This not only greatly speeds up the normal

series of events but may actually be deceptive with regard to certain particulars. We have all observed in ordinary movies the spokes of a wagon wheel moving in the wrong direction, which would not happen if the individual frames had been exposed with less time interval between them. The actual speed of chromosome movements is so slow that the motion itself is not detectable as movement; hence, to look on these as jerking or tugging motions, as we commonly use these terms, is hardly justified.

3) A single chromatid that has two attachments to the spindle—that is, a dicentric—usually shows during anaphase a decreased diameter in one or more regions before a break occurs at some point. This has every appearance of a stretching effect with the separating force applied at the centromeres. It does not tell us, however, whether the force is a pulling or pushing one.

An explanation of anaphase chromosome movement that postulates a pulling or spindle fiber contraction force must assume that the poles are fixed; otherwise spindle contraction would be as likely to move the poles toward the chromosomes as the chromosomes toward the poles. The poles could be held apart either by astral rays connecting each pole of the spindle to the adjacent region of the cell membrane or by the so-called "continuous fibers" of the spindle extending from pole to pole. Astral rays, few in number and very delicate, are demonstrable with the microdissection needle in living neuroblasts and can be seen occasionally in fixed and stained neuroblasts. That they are not essential to anaphase, however, is proved by the fact that anaphase movement of the chromosomes continues as the microneedle rotates the spindle through 180 degrees to reverse it end for end, during which astral connections between poles and cell membrane cannot remain intact (*8*). Continuous fibers can sometimes be seen in fixed and stained preparations, but in the living neuroblast the whole interzonal region, except for the interzonal fibers connecting the distal ends of sister chromosomes, seems quite fluid, and it is not possible with the microneedle to demonstrate the presence of any continuous fibers extending from pole to pole.

The hypothesis of a pushing rather than pulling action is also supported by the effect of holding a needle tightly pressed against a metaphase cell adjacent to a pole of the spindle. Cell elongation at anaphase is partially prevented, but eventually the spindle and attached chromosomes, pressed tightly against the cell membrane, move some distance past one side of the needle (*8*).

Bělař (*10*), on the basis of extensive observations of meiosis in cells that had been shrunken or swollen by hypertonic or hypotonic salt solutions, respectively, concluded that after the initial separation of the chromatids the subsequent movement apart was the result of elongation of the *Stemmkörper*—that is, the part of the spindle situated between the separating centromere groups. In the grasshopper neuroblast, however, this is not the semisolid structure apparently visualized by Bělař in the grasshopper spermatocyte, but is a highly fluid region that I hesitate to call part of the spindle. By middle anaphase, all that resembles the original spindle is a semisolid cone between each pole and the set of daughter chromosomes approaching it. Liquefaction of the bulk of the spindle has taken place. Except for the mitochondria that have moved in from the cytosome, the only nonfluid structures between the separating sets of chromosomes are the interzonal fibers connecting distal ends of sister chromosomes. I suggested in a recent paper (*8*) that elongation of these fibers was probably responsible for much of the anaphase movement of sister chromosomes. Though the apparent "stretching" of dicentric chromosomes during anaphase and the movement to one pole of unpaired chromosomes at meiosis appear at present to militate against the universal applicability of this hypothesis, I believe the evidence at present for pushing is better than that for pulling apart of daughter chromosomes, at least in all except the initial stage of anaphase separation.

Summary

My conclusions, which, I confess, are tentative and based mainly on studies of one kind of cell, the grasshopper neuroblast, may be summarized as follows.

The late prophase orientation of the chromosomes is a carry-over from the late telophase orientation. It is apparently maintained by means of the centromeres, which appear to be attached within a limited region of the nucleus throughout telophase, interphase, and prophase.

Metaphase orientation of the chromosomes may be explained as the resultant of two forces: a force involving the centromere and spindle, which is responsible for keeping the centromeres in the equatorial plane of the spindle, and a repulsion force involving the noncentromeric portion of the chromosomes, which results in a tendency toward uniform spacing of the chromosomes outside the spindle.

Anaphase separation of sister chromatids and their subsequent movement toward the poles of the spindle involves at least four distinct phases: (i) the initial poleward movement of the centromeres, which may be due to intrinsic repulsion or to a force acting between spindle and centromeres that produces an angle of almost 90 degrees between the separated and unseparated portions of the chromatids; (ii) the autonomous separation of the noncentromeric part of the chromosome; (iii) elongation of the spindle, beginning just after the sister chromatids are separated proximally and ending when the longer chromatids are about to lose contact distally; and (iv) the later movement apart of the daughter chromosomes, probably resulting from a pushing force exerted by elongation of the interzonal fibers.

References and Notes

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There is, I think, nothing in the world more futile than the attempt to find out how a task should be done when one has not yet decided what the task is.—ALEXANDER MEIKLEJOHN, in *Education Between Two Worlds.*