Mitotic Cell Studies Based on *in vivo* Observations VIII.

The evolution of mitotic spindles in eukaryota: a negation of the breakdown of the nuclear membrane

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Received September 17, 1974

The mitotic spindle is an absolutely necessary intracellular organelle for cell proliferation providing each daughter nucleus with an exact copy of the genes present in a mother cell. Each daughter nucleus then separates into two daughter cells by cytokinesis. For the accomplishment of nuclear division each cell employs chromosomes and a spindle body.

Current cytology books deal with studies on the chromosomes as the main subject. The description of the mitotic spindles is rather *jejune* and imperfect, because the spindle contains still, many enigmas and controversial interpretations. One of the most important of these is that in the higher plant and animal cells the nuclear membrane is axiomatically believed to break down before spindle formation, despite the fact that the spindles in living cells are enveloped in a spindle membrane continuously derived from the nuclear membrane (Wada 1950, 1966). The cytokinesis is explained by a cell plate formation in plant cells and by a cleavage furrow formation in animal cells.

In this paper the author explains the reason and process by which the nuclear membrane in higher plant and animal cells, when fixed, is artificially broken down before spindle formation. In comparison with intranuclear spindles in protista, including fungi and algae, and also with the spindles in *in vivo* observations, the author points out that the description of the breakdown of the nuclear membrane before spindle formation in higher plant and animal cells is a gross error induced by fixation cytology including electron microscope studies; and cytology books still describe this error after nearly a century.

To many unsolved and controversial problems on mitosis based on the misinterpretation of the nuclear membrane in mitosis, the author presents answers and proposes a new interpretation viz. that the nucleus can alter continuously from a metabolic state into a karyokinetic one, and the latter state is represented by the karyokinetic spindle itself.

Detailed exposition

1. Unsolved problems in the mitosis described in current cytology books

The most important and fundamental problem which faces present mitotic cell

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studies is 1) to inquire concretely into the continuous morphological changes of mitotic figures from prophase to the end of telophase, and 2) to establish the cause and effect relations among them. However, current cytology books¹ as a matter of course give no answers to the problems which should have been elucidated morphologically before modern biological analyses of a biochemical as well as a biophysical nature were, as it were, enforced on mitotic performances.

a. *Breakdown of the nuclear membrane before spindle formation in higher plant and animal cells*

This concept is generally accepted for mitosis in metazoa and metaphyta. However, no explanation has ever been offered to clarify the biological significance of this event in contrast with the intranuclear spindle mitosis in protista including fungi and algae (Fig. 1). Yet cytology books have hesitated to cite the various findings which have demonstrated the continuous transformation of the nuclear membrane into the spindle membrane as obtained in *in vivo* observations (Fig. 2) and also in fixed preparations (Figs. 1 j-1) when treated with improved fixatives in higher plant, animal, and cancer cells (Wada 1966, 1970b, 1972b).

Cytology books have strenuously insisted upon the breakdown of the nuclear membrane as seen on the basis of fixation cytology. Thus, young cytologists have been, as it were, left in the dark with regard to the search for factual truth in the behavior of the nuclear membrane in mitosis. Cytology books have also neglected classical but important descriptions relative to the anastral type of nuclear division in the animal kingdom in which karyokinetic spindles are formed by a direct transformation of the nuclear substance (Wilson 1928, p. 150).

b. *Spindle fibers and spindle background substance*

The presence of kinetochore fibers (viz. chromosomal fibers, traction fibers in classical cytology) are generally confirmed morphologically and functionally. Other fibers, such as continuous fibers, sheath fibers, connecting fibers, and also microtubules are nothing but various coagulated features of spindle background substances, the attractoplasm, mainly induced by differences of fixatives, magnifications, and positions in a spindle body. No intrinsic differences among them are recognizable in *in vivo* observations (Wada 1950) and also in spindle birefringence (Wada 1966 p. 36).

Current cytology books describe nothing of the spindle background substance in which chromosomes and kinetochore fibers are embedded. Also, neither the origin and state of the spindle background substance in prophase nor its destiny in telophase are explained.

c. *The revelation process and biological significance of fusiform of mitotic spindles in eukaryota*

All the mitotic spindles in unicellular and multicellular organisms fundamentally show the fusiform (spindle form). However, neither the cause and processes of the

¹ For instance: Cell Biology (1970) 5th ed. E. D. P. Robertis, W. W. Nowinski and F. S. Saez; W. B. Saunders Company, Philadelphia, London, and Tronto. Grundlagen der Cytologie (1973) ed. G. C. Hirsch, H. Ruska and P. Sitte; VER G. Fischer, Jena. Text-Book of Cytology (1974) 2nd ed. W. V. Brown and F. M. Bertke; The C. V. Mosby Company, Saint Louis, U.S.A.

Fig. 1. Showing evolution of mitotic spindles in eukaryota. a-e. Intranuclear spindle (Type I) of meiosis in young basidium of *Mycena haematopoda* Pers. Agaricaceae (Wakayama 1930). a-b, diakinesis. c, metaphase. d, anaphase. e, early telophase. $\times 3800$. f-i. Intranuclear spindle (Type II) in antheridium of *Sargassum Horneri* (Courtesy of Dr. Inoh). f, resting stage. g, synapsis stage. h, metaphase, side view. Centrosomes adsorb both spindle poles. i, anaphase. $\times 2000$. j-l. Cormophyte spindle (anastral type) showing spindle membrane in root-tip cells of *Vicia faba* fixed with Cd-fixative. Arrows indicate positions of membrane (Wada *et al.* 1963). j, a slight protruding of nuclear membrane in late prophase. k, spindle membrane in late prometaphase. l, spindle membrane in mid-telophase. $\times 1500$.

fusiform formation in the appearance of karyokinetic spindles, nor the necessity and biological significance of this form for the spindle activity have ever been explained in cytology books.

d. *Mitotic performance without centrioles in higher plant cells*

In the mitosis of animal cells, centrioles (centrosomes in classical cytology) are explained as the kinetic center of spindle fiber formation. In higher plant cells, however, centrioles including aster rays completely disappear from the cell (Wada 1969b, 1972a). And yet no fundamental differences are found between the karyokinesis in plant cells and animal cells with regard to the formation and activity of meta- and anaphase spindles.

Nevertheless, no cytology books known to this author have presumed to revise the concept of the centriole spindle fiber formation.

e. *Paucity of comparative studies on the mitosis among protista, higher and lower plants and animals*

According to the morphological differences of spindles in reference to the protista, the animals, and the higher plants, three categories of mitosis are described viz. 1) intranuclear spindle mitosis, 2) centrosome spindle mitosis, and 3) cormophyte spindle mitosis (Geitler 1934). The performance of mitosis in protista has been dealt with as an exceptional case and reported generally in specialized journals.

Cytologists working on mitoses in higher plant and animal cells seem indifferent to the mitosis in protista. However, the primitive types of mitosis in protista are essential to understand the origin of spindles in presently existing higher plant and animal cells which would have developed and differentiated on the principle of teleonomy (Monod 1970) in the course of evolution in eukaryota.

f. *Materials used for spindle studies unfortuitous for wide selection*

Since the end of the nineteenth century up to this date, animal cytologists have habitually used the cell divisions of sea urchin egg cells as material to study mitosis. The conspicuous centriole-aster system in sea urchin egg cells conceals completely the contour of polar regions of the metaphase spindles (Wada *et al.* 1964) (Fig. 4a). The sea urchin egg may be not only an inadequate material but also a rather rare case among the enormous number of species in the animal kingdom, should the frequency of such species, whose centriole-aster system is as conspicuous as that of sea urchin groups, be computed statistically.

The performance of cell division in culture cells reveals the sudden but continuous transformation of prometaphase nuclei directly into metaphase spindles in cine-microphotographs. This phenomenal fact, however, has occasioned no new interpretations of the spindle formation among animal cytologists.

Although sea urchin groups apparently surpass all other research materials in ease to obtain and in the quantity of observation data since the days of the establishment of cytology, they cannot be considered to be a scientifically selected material for studying mitosis. It is common sense in modern science to select that material which has a simpler organization for analyzing any complicated phenomena such as the mechanism of mitosis. Higher plant cell mitosis having no centriole-aster system is decidedly superior to animal cell mitosis which has a complicated

cytoplasmic organization (Wada 1972a).

Nevertheless, cytology publications describe mitosis in sea urchin groups as a model of mitosis. Some animal cytologists suppose the presence of pre-centriole-like inclusions in higher plant cells (Luykx 1970). This may be an erroneous explanation occasioned by prejudice in favor of the so-called mitotic apparatus (Mazia 1961). For over one hundred years, mitoses in sea urchin groups have been studied by animal cytologists and yet *many fundamental problems* in this mitosis are left unsolved as pointed out in this chapter. On the contrary, it is well known that the selected research material, *Drosophila*, has greatly contributed in the establishment of genetic principles within the half century.

Many of the unsolved problems (a-e) in mitosis as mentioned above are apparently due to the lack of *in vivo* observations and to reliance on fixed figures even including electron microscope studies, although observations of fixed figures while essential are unavoidable in masking cytological facts. Cytologists are always compelled to judge and interpret any continuous morphological changes in living cells from a series of separately fixed states of killed cells.

2. Merit and demerit of fixation cytology in the study of mitosis

a. *Artifacts and in vivo observations*

Current cytology books describe and explain various fixed figures of mitosis as if they were in the same state in the living cells without indicating the supplementary fact that any kind of mitoses is manifested only in a state of approximately 70–80% water content (Wada 1970b). As far as mitoses are concerned, descriptions of current cytology books, especially those of electron microscope studies, are apparently of *fixation cytology* but not of *cell biology*. In this way, considerations of artifact problems and the importance of *in vivo* observations have been shunted away from the attention of young cytologists. They learn nothing about the dynamic state of mitotic cells which are intrinsically different from fixed figures illustrated in cytology books.

On the other hand, the author does not grudge due appreciation for the merits of fixation cytology which have given various contributions to the *static morphology* of cell organelles, such as chromosomes, mitochondria, ribosomes, etc., and he praises its meritorious contributions to advances in cytology, cytogenetics and cognate areas. Mitosis, however, is a manifestation of continuous *dynamic morphology* of intranuclear organelles in living cells and cannot be alone and finally elucidated with fixed figures including electron microscope studies.

b. *Phase contrast microscope*

The development of phase contrast microscopes has brought remarkable assistance to *in vivo* observations of mitotic cells, and especially of chromosome behavior in animal cells. However, to obtain clear optical images it is necessary to press mitotic cells and to bring the object to a thin focal plane. This unnatural treatment of the mitotic cells disturbs the intrinsic contour of karyokinetic spindles and causes misinterpretations about the determination of the presence of a thin surface membrane, or of granules being adsorbed onto the membrane (Wada 1970b) (Fig. 2).

c. Observation media

Staminal hair cells of *Tradescantia* used for *in vivo* observations are generally observed in media of isotonic or slightly hypotonic sucrose solutions. However, the living state of hair cells in media are not always healthy and become unstable showing various abnormalities.

By covering hair cells with a thin agar plate containing 2% sucrose, they enter mitosis normally, similar to many animal cells in culture entering mitosis by the ad-

Fig. 2. Time lapse photomicrographs of endosperm cell mitosis in *Zephyranthes candida* cultured in a medium of 1% sucrose and 0.5% agar. Photographed with ordinary light microscope without compressing the cell to a thin disc and focused on spindle membrane and granules (g_1 and g_2) adsorbed outside of nuclear membrane. Arrows indicate positions of membrane. a'-c'. Drawings of above photographs. a, late prophase. b, prometaphase. Spindle substance (so-called clear zone by Bajer 1957) appears in nuclear cavity. c, early anaphase. Granules adsorbed outside the prophase nuclear membrane remain on the same position of spindle membrane showing no breakdown of nuclear membrane in living cells (Wada and Kusunoki 1964a).

dition of phytohemagglutinin (Wada 1943). Applying the thin agar plate method, it becomes possible to trace continuously the appearance of mitotic aberrations (induced experimentally by chemicals, X-rays, UV microbeam irradiations or with other physical treatments) and also their recovery processes (Wada 1966).

d. *In vivo* observations of endosperm cell mitosis

Modifying this agar thin plate method, Bajer and Molé-Bajer (1954) have succeeded in observing the mitosis of young endosperm cells in a living state by the

glucose agar method. This technique has greatly contributed to open the possibility of observing mitosis in various phanerogamous plants in a living state.

Bajer and Molé-Bajer (1953) have reported a finding from their *in vivo* observations—that the spindles in somatic cells of *Hymenophyllum* are nuclear in origin. However, from the results of *in vivo* observations on endosperm cell mitoses in *Haemanthus*, Bajer (1957) has concluded that the breakdown of the nuclear membrane at the end of prophase and the appearance of a clear zone (initial substance of the spindle) take place outside the nuclear cavity. This sudden change in his interpretation has been accepted by cytology books which hold fast to the classical spindle formation theory of the breakdown of the nuclear membrane disregarding the different fact in *Hymenophyllum* cell mitosis.

After Bajer's *in vivo* observation technique, the author has re-investigated endosperm cell mitoses in various phanerogamous plants with special reference to the behavior of the nuclear membrane and could find neither any evidence to confirm the breakdown of the nuclear membrane nor the appearance of spindle substance outside the nuclear cavity (Wada and Kusunoki 1964) (Fig. 2). Not only in endosperm cells but also in somatic as well as meiotic cells, the spindle substance is nuclear in origin and enveloped in the spindle membrane (Wada 1966).

From the results of *in vivo* observations in plant and animal cells including cancer cells, the author has reached the conclusion that Fujii's atractoplasm theory is true for all the mitosis in eukaryota.

e. *Birefringence of mitotic spindles*

Schmidt (1939) was the first to report a strong birefringence in the spindles of fixed plant mitotic cells under the polarization microscope. In living cells of plants and animals, the chromosomes, spindles, and many other intracellular organelles are in a hydrophilic state and it is difficult to observe their clear-cut contours under the microscope.

Inoué (1953) had overcome this optical difficulty technically and succeeded in demonstrating the birefringence of mitotic spindles in living cells under the polarization microscope. His findings were the first to provide a clue for analysing the fine structure of living mitotic spindles at the molecular level. Because of this birefringence of the spindles, it is generally accepted that the molecules in the spindles arrange themselves parallel to each other forming an optically positive anisotropic arrangement to the spindle axis. In the analysis of spindle structures by birefringence extinction experiments, however, Inoué (1967, 1974) has accepted the interpretation of the breakdown of the nuclear membrane and the presence of not only kinetochore fibers but also of continuous fibers and sheath fibers, just as in classical fixation cytology.

Both in plant and animal cells, the strong birefringence of kinetochore fibers would appear in an evenly illuminated but weak birefringence of the spindle background substance, viz. the atractoplasm (Fig. 3). However, no birefringence of other fibers described in fixation cytology are in any way distinguishable. In living cells, the evenly illuminated birefringence of the prometaphase spindle (Figs. 3a, e) apparently denies the presence of the so-called continuous fibers which connect pole to pole (Wada 1966 p. 35). If continuous fibers were to exist, the spindle poles would

have to shine always stronger than the equatorial region due to the birefringent images of converged continuous fibers.

In plant cell mitoses, the birefringence of the cell plate appears in telophase. This always shows optically negative birefringence in contrast to the spindle axis (Fig. 3f).

Fig. 3. Birefringence of mitotic spindles in living cells showing strongly illuminated kinetochore fibers and only weakly luminous spindle background fibrils. a-c. Meiosis of spermatocyte in *Chrysochraon japonicus* followed by cine-photomicrography (Izutsu *et al.* 1974). a, prometaphase spindle showing birefringence of evenly luminous spindle background fibrils. b, late metaphase showing a few kinetochore fibers which are illuminated more strongly than the spindle background fibrils. c, mid-anaphase showing shining spindle poles due to converged kinetochore fiber birefringence and weak luminous birefringence of spindle background fibrils between the poles (Courtesy of Dr. K. Izutsu). d-f. Time lapse photomicrographs of an endosperm cell in *Zeaphyranthes candida* cultured in endosperm fluid (Niitsu and Hanaoka 1972). d, early anaphase spindle photographed with ordinary light microscope. e and f. Same cell photographed with polarization microscope. e, short, bright kinetochore fibers growing from kinetochores toward spindle poles in weakly luminous spindle background fibrils. f, mid-telophase showing negative birefringence of cell plate (Courtesy of Dr. T. Niitsu).

3. The independence of both nucleus and mitotic spindle from the cytoplasm, predicted and organized by gene information

It must have been an epoch-making event when one of the prokaryotic living

things had accomplished the organization of the nucleus in their protoplast in the course of evolution. Modern genetics states that any kind of organ in living things is predicted and built up autonomously and differentiates teleonomously through information in the genes (Monod 1970). Accordingly, it is natural to assume that the nucleus and the mitotic spindle would not be organized unless the advent of a particular gene or genes should have taken place in the course of gene evolution.

The essential characteristic of eukaryota which distinguishes them from prokaryota is to assemble scattered genetic material (chromosome DNA), nucleolar RNA, and nuclear sap proteins in one nucleus and to envelope them in a nuclear membrane. The first gene evolution which eukaryota would have acquired might be a gene or genes which could give information to construct the nuclear envelope. Then chromosome DNA, nucleolar RNA, and nuclear sap proteins might have been housed in a nuclear cavity. This new type of living thing must have been a predecessor of the eukaryotic organisms.

Fig. 4. Spindle membranes in animal cell mitosis in fixed preparations. Arrows indicate positions of spindle membrane. a, metaphase spindle of egg cell mitosis in *Hemacentrotus pulcherrimus* showing presence of spindle membrane (Wada *et al.* 1964). b-c. Meiosis of spermatocytes in *Chloelitis geniculaributs* (Nakao *et al.* 1968). b, a side view of spindle in metaphase II. Centrioles adsorbed outside of spindle membrane at both poles. c, spindle membrane in metaphase I. Centrioles lying inside of cell membrane apart from spindle poles. a-c, fixed with Bouin's fluid after pretreatment with 1/10 M MnSO_4 solution.

Consequently, so long as a cell or an individual belong to the eukaryota, it is a genetically gifted characteristic that the nucleus is morphologically independent of the cytoplasm through the presence of a nuclear membrane, irrespective of whether the nucleus is in a metabolic state or in a karyokinetic state. In this way, each nucleus can ensure the purity of genes and facilitate gene activity in a metabolic as well as in a mitotic state of the cell.

The presence of a nuclear membrane in the mitosis of protista, including the fungi (Figs. 1a-e) and algae (Figs. 1f-i) is generally observable even in fixed preparations. In this case, their nuclear membranes, in which the metaphase spindles are enveloped, are strong enough to resist the coagulation shock induced by routine

fixatives prescribed for chromosome studies. In metazoa and metaphyta, due to the enlargement of the nuclear volume, when a metabolic nucleus becomes transfigured into a karyokinetic one, a considerable tension would have to be exerted on the nuclear membrane and it would enter into a strained state and become fragile in the presence of fixation shock, when fixed (Wada 1950).

Although by *in vivo* observations and also by treatment with improved fixatives prescribed for the conservation of the spindle membrane and the spindle background fibrils (Wada and Fukunaga 1957, Wada *et al.* 1963), the presence of the nuclear membrane throughout the mitotic cycle of higher plant and animal cells has been demonstrated both in living and fixed cells, e. g. in the *in vivo* observations of *Tradescantia* hair cells, young prothallium cells of *Osmunda japonica*, young stipular cells of *Vicia faba*, and endosperm cells of *Zephyranthes candida* (Fig. 2), and in the fixed preparations of root-tip cells of *Vicia faba* (Figs. 1j-l) and *Allium cepa*, pollen mother cells of *Tradescantia*, *Hosta*, *Zephyranthes*, spermatocytes of *Chloelitis geniculaributs* (Figs. 4b-c), egg cells of *Hemicentrotus pulcherrimus* (Fig. 4a) and Ehrlich ascites tumor cells¹.

All these examples demonstrated the continuous morphological transformation of the prophase nuclear membrane into the metaphase spindle membrane. Writing from a cell-biological standpoint, a metaphase spindle is nothing but a spindle-shaped nucleus enveloped in a biological membrane (a nuclear membrane) for preparing the poleward movement of separate chromosomes in anaphase; the spindle membrane and the atractoplasm at metaphase then correspond to the nuclear membrane and the nuclear sap of the metabolic nucleus respectively.

4. Atractoplasm

From the results of his *in vivo* observations of meiosis in *Tradescantia* pollen mother cells, Fujii (1931) expressed the opinion that the atractoplasm, viz. spindle background substance, might originate from the karyolymph. Detailed explanation of the atractoplasm theory is given in "Analysis of Mitosis" (Wada 1966). As already mentioned in the foregoing chapter, the concept of the atractoplasm is true for all kinds of protista, metazoa and metaphyta including fungi and algae.

a. *Transfiguration of a metabolic nucleus into a karyokinetic one (metaphase spindle) as evidenced from the evolution of mitotic spindles*

Since the establishment of cytology, nuclear sap in metabolic nuclei and also in early prophase nuclei is described as a fine network or an amorphous structure in fixed preparations and as structureless solution in living cells. From these data and from staining experiments, the nuclear sap is explained as a protein colloidal solution in which chromosomes and nucleoli are embedded.

Under the polarization microscope, when once a prophase nucleus enters into metaphase, a birefringence of the metaphase spindle appears at the position of the prophase nucleus. This optical property of metaphase spindles may not be confined, however, to the spindles of higher plants and animals alone. In the case of intranuclear spindles of protista including the fungi and algae, the birefringence of metaphase

¹ All these data and photographs have been published in Cytologia (1935-1964) and collectively described in "Analysis of Mitosis" (Wada 1966).

spindles would have to appear in their nuclear cavities, when observed under the polarization microscope, although it may be highly difficult to catch the birefringence of the nuclear membrane. From this consideration it seems to be a most natural interpretation that the spindle background substance, the attractoplasm, is nuclear in origin irrespective of whether in higher and lower plants and animals. If further conjecture would be allowed, the author will express without hesitation the opinion that the birefringence of metaphase spindles may be induced by the transformation¹ of dispersed globular proteins in the nuclear sap into fibrous ones in the attractoplasm.

Table 1. Surface area and volume of a dividing nucleus (a-h) calculated as spheroids on the basis of outlines in a living staminal hair cell of *Tradescantia reflexa*. a, early prophase (14: 08). b, mid-prophase (14: 35). c, late prophase (15: 35). d, polar cap stage (16: 20). e, prometaphase (16: 25). f, metaphase (16: 45). g, late anaphase (17: 03). h, mid-telophase (17: 15). Numerals in parentheses indicate the hours when photographed (Wada 1966).

Stage of mitosis Nucleus and spindle	a	b	c	d	e	f	g	h

Surface area (μ^2)	1685.9	2296.2	2331.6	3041.4	3321.2	3592.7	3960.2	3297.6
Relative value of surface area	100.0	136.2	138.3	180.4	197.0	213.1	234.9	195.6
Volume (μ^3)	6333.5	9500.3	10038.6	14035.0	16967.4	19488.2	21818.9	15979.4
Relative value of volume	100.0	150.0	158.5	221.6	267.9	307.7	344.5	252.3

1. *Intranuclear spindles (Type I)*: There are two kinds of intranuclear spindles in protista including the fungi and algae. In the case of fungi, to transport a small number of minute chromosomes to each spindle pole it would not be necessary to transform all the nuclear sap into the attractoplasm (Wada 1972b). Accordingly, a slender spindle would appear in the nuclear sap which surrounds the spindle (Wakayama 1930) (Figs. 1a-e). Therefore, the nuclear membrane remains as it is in prophase. This may be a prototype of the mitotic spindle in the course of evolution and it also may demonstrate the nuclear origin of the spindle background substance, the attractoplasm.

2. *Intranuclear spindles (Type II)*: In this type of intranuclear spindle (Figs.

¹ In this paper as a matter of convenience, the author uses the word "transformation" for the phenomena by which individual molecules or molecule groups in an isotropic state optically change into an anisotropic state. Concerning transition processes of globular proteins into fibrous ones at molecular level, the author will touch on them in the concluding remarks.

If-i), the nuclear sap becomes wholly transformed into the atractoplasm and remains no longer in the nuclear cavity. Therefore, when fixed, such spindles seemingly show no apparent differences from those in higher plant and animal cells except for the presence of their spindle membrane. In this case, the nuclear volume, when transformed into a metaphase spindle, seems to change very little or not at all. Accordingly, no tension seems to be exerted on the membrane which may be able to endure the coagulation shock induced by fixatives, in just the same way as the prophase nuclear membrane does.

3. *Spindles in higher plant and animal cells:* In higher plant and animal cell mitoses, when prophase nuclei become transformed into metaphase spindles, a remarkable increase of membrane surface area is calculated in general (Table 1). Accordingly, a considerable tension may fall on the surface membrane of metaphase and anaphase spindles and these then may enter into a strained state in comparison with the membranes of prophase nuclei. This expanded state of the spindle membrane had been demonstrated by the widely scattered membrane pores observed under the freeze etching electron microscope (Sprey 1972). This strained state of the spindle membrane is considered to be the main cause of its breakdown into fragments by sudden coagulation shocks, when fixed (Wada 1950, 1972b). No mechanical strength of the spindle membrane may be demanded to facilitate the spindle activity and to keep the morphological independence of the atractoplasm from the cytoplasm (Wada 1966, 1972b). However, it is a primary activity of the metaphase spindle which prepares space enough to arrange chromosomes at the equator, to transport separate chromosomes toward spindle poles and to set apart the daughter nuclei.

Summarizing the explanations mentioned above, the relations between nuclei in a metabolic state and in a karyokinetic state in eukaryota can be expressed in a simplified schema as follows:

Metabolic (resting) nucleus, spherical in form composed of 1) chromonemata, 2) nucleoli, 3) nuclear sap (dispersed globular proteins), and 4) nuclear membrane.

Karyokinetic nucleus (metaphase spindle), spindle-shape in form composed of 1) chromosomes, 2) kinetochore fibers, 3) atractoplasm (arranged fibrous proteins), and 4) nuclear membrane (a strained state due to enlargement of the nuclear volume in the case of higher plant and animal cells).

b. *An assumption about the constitution of the atractoplasm*

From the results of *in vivo* observations on the continuous morphological changes of prophase nucleus into metaphase spindle in higher plant and animal cells and from comparative studies on intranuclear spindles, it needs no argument to assume that the birefringence of metaphase spindles may be caused by the transformation of dispersed globular proteins in nuclear sap into fibrous proteins in metaphase spindles. Because no nuclear sap in prophase shows birefringence, and because, when once metaphase spindles appear, spindle bodies show birefringence, at first uniformly, (Figs. 3a, e) succeeded by birefringent figures of the kinetochore fibers from each kinetochore of chromosomes toward both spindle poles (Figs. 3a, e).

This phenomenal fact reveals that the dispersed proteins in prophase nuclear sap would be no longer globular but fibrous, and would arrange themselves parallel to the spindle axis under the influence of polarity. Accordingly, the appearance of metaphase spindle birefringence needs neither the activity of centrioles nor any kind of proteins present outside the nuclear cavity.

This explanation of the developmental process of metaphase spindles is valid in all eukaryota. It differs, however, fundamentally from the explanations described in current cytology books. This discrepancy may be due to the difference of interpretations based on *in vivo* observations and on fixation cytology relative to the following: 1) a denial of the breakdown of nuclear membrane, 2) results of comparative studies on the spindle morphology among protista, metazoa, and metaphyta, 3) consideration of the differentiation of mitotic spindles toward teleonomy in the course of evolution in eukaryota, and 4) genetic consideration of the morphogenesis of the nuclear membrane and mitotic spindles being predicted and controlled by gene information.

Applying improved fixatives for spindle fixation, Satô (1958, 1959, 1960) has succeeded in demonstrating the presence of dispersed globular particles in prometaphase and of fibrous ones in metaphase as spindle background substance under the electron microscope. His findings (1960, Figs. 2-8) are enough to support the present author's assumption on the transformation of nuclear sap in prophase into spindle background fibrils in metaphase.

The development and constitution of kinetochore fibers in the mitotic spindle are explained in the next chapter.

c. *Polarity*

Polarity in biology is a fundamental but difficult problem. It predicts and precedes the morphogenesis of tissues and organs in eukaryota and has various relations not only in cytology but also to either embryology or to ontogeny. The parallel arrangement of all transformed fibrous proteins to the spindle axis is one of the manifestations of polarity which is *apriori* in the cell.

d. *Formation of the fusiform of metaphase spindle*

It is a well known fact that, fibrous molecules or molecule groups arrange themselves parallel to a common orientation and thus would often make a tactoid as a whole, irrespective of whether in living or non-living material. In the case of mitotic spindles, the fibrous proteins arranged under the influence of cell polarity may make a tactoid by themselves. This developmental process of metaphase spindles reveals clearly the reason why metaphase spindles in eukaryota always show a fusiform.

Colchicine and mercaptoethanol are well known agents which can reversibly suppress the spindle activity. Metaphase spindles treated with colchicine lose their fusiform and become circular, but still maintain their surface membrane which is traceable in *in vivo* observations of *Tradescantia* hair cells (Wada 1940). In electron microscope studies, Satô (1970) reported that the spindle background fibrils and kinetochore fibers in deformed functionless metaphase spindles of pollen mother cells in *Lilium* appear under the electron microscope as irregularly dispersed

fine granules or short rows of granules corresponding to their previous structures, when treated with colchicine and mercaptoethanol. These findings give strong support to the conclusion that the metaphase spindle is functional when they are tactoid or fusiform. Satô's experiments also have revealed that each kinetochore fiber might have been composed of a densely arranged bundle of spindle background fibrils.

e. *Role of nucleoli during mitosis*

The disappearance of nucleoli at the time of spindle appearance is a well known fact both in fixed preparations and in *in vivo* observations. With regard to nucleoli, current cytology books describe exclusively those interpretations based on fixed figures in metabolic nuclei and deal with ribonucleoproteins, ribosomal RNA synthesis or nucleolar organizers and related phenomena as the role of nucleoli.

However, cytology books give no explanation for the cause and effect relations of why nucleoli have to disappear before spindle formation, why their RNA would have to be released during meta- and anaphase spindle activity, and why the reappearance of nucleoli in each daughter nucleus can take place in telophase. From the results of repeated *in vivo* observations and of experiments on the behavior of nucleoli in *Tradescantia* hair cells (Wada 1936a), the author does not hesitate to propose the following working hypothesis (Wada 1966 p. 114). By disintegration of nucleoli before spindle formation, released nucleolar RNA would play the role of an energy source or polymerization agent for the transformation of nuclear sap globular proteins into fibrous ones in prometaphase and *vice versa* in telophase (Wada 1966).

The globular proteins in the daughter nuclear sap may be supplied by retransformation or depolymerization of fibrous proteins in the spindle. The released RNA may reform new nucleoli at first, mainly by accumulating around nucleolar organizers in telophase. Thus the author ventures to say that the nucleolus may be a reservoir of particular differentiated RNA prepared for the transformation of prophase nuclear sap into the atractoplasm in the course of evolution in eukaryota.

5. Formation of kinetochore fibers

According to the atractoplasm theory, kinetochore fibers (chromosomal fibers or traction fibers in classical cytology, etc.) are made up by binding the spindle background fibrils into a fiber, set with the kinetochore substance exuded from the kinetochore at each chromosome (Wada 1966 p. 101). This interpretation is most natural and valid based on the constitution of the metaphase spindle not only in higher plants and animals but also in protista including fungi and algae. In addition neither centrioles, which are absent in higher plant cells, nor any kind of monomers of unknown entity are required to form kinetochore fibers.

The kinetochore substance exuded from a kinetochore site at each chromosome may penetrate into interfibrillar spaces probably by a capillary phenomenon along the tracks of fibrils, and may also be able to reach each spindle pole without error. During the flux of the kinetochore substance, all the fibrils on the track get set into a bundle and finish the formation of each kinetochore fiber between the kinetochore and corresponding spindle pole.

As facts of phenomena it is well known that in fixed and also in birefringent

figures the kinetochore fibers of large size appear to converge toward the kinetochore of each chromosome and to diverge toward the polar regions of the spindles. This contour of each fiber may apparently correspond to the locus along which the fluid kinetochore substance would have flowed out from each kinetochore (Wada 1950, 1966). Recently, Niitsu *et al.* (1972) strongly supported this explanation of the kinetochore fiber formation by their investigations in *Ornithogalum* pollen mitosis treated with cycloheximide.

Fig. 5. Effects of cycloheximide of 1 ppm solution on the development of kinetochore fibers in pollen mitosis of *Ornithogalum virens* in living cells (Niitsu *et al.* 1972). Abscisa: elapse of time (minutes) after cell culture. Ordinate: percentage of mitotic cells at each stage in the total number of cells observed. PM: prometaphase, M: metaphase, A: anaphase, and T: telophase. a, normal mitotic frequency of the second pollen grain mitosis after prometaphase. b: mitotic frequency in cycloheximide solution (1 ppm). The greatest effect appears on prometaphase cells by suppression of kinetochore fiber development. c-d: Treatment of cycloheximide began at different times in culture. Numerals connected with a broad line in abscissa indicate the time of cycloheximide treatment. c: duration of treatment was confined to 0-20 minutes. Prometaphase cells were remarkably arrested. d: 40-60 minutes. Mitotic cells already at anaphase, when treatment began, show no effect by cycloheximide treatment (Courtesy of Dr. T. Niitsu).

According to their findings, a cycloheximide solution of 1 ppm does not suppress mitosis completely: it retards the process of mitotic stages, particularly by suppressing the entrance into metaphase. In the mitotic cells treated with this chemical, kinetochore fibers do not appear in meta- or anaphase spindles, on which occasions it is ineffective, being a passing phenomenon. The arrested mitotic cells can enter mitotic activity again with the termination of the cycloheximide treatment. Accord

ingly, the delayed time in each mitotic stage exactly corresponds to the time of cycloheximide treatment (Fig. 5).

From the results of their studies, it can be concluded that the substance exuded from each kinetochore and acting to set the spindle background fibrils into a fiber is a kind of protein. Also judging from the morphogenesis of kinetochore fibers mentioned above, both the repair of the partial damage of kinetochore fibers (Forer 1965) and the arresting of the kinetochore fiber formation, and accordingly the arresting of chromosome movement (Takeda and Izutsu 1960) induced by UV microbeam irradiation, are not difficult to understand.

6. Biological significance of electron microscopic figures in the elucidation of mitotic performance

Since the introduction of electron microscope studies on mitotic cells, the findings on microtubules have been awaited to elucidate the mechanism of mitosis in place of the so-called spindle fiber explanation. As already repeatedly explained (Wada 1970b, 1972b), mitosis is a continuation of dynamic morphological changes of intranuclear substances positively manifested *in se* in living cells having approximately 70–80% of water content. In spite of this fact, at present, no electron microscopes possess powers to visualize any structures of the spindles in a living state of cells.

With the exceptions of kinetochore fibers, the so-called continuous fibers, sheath fibers, connecting fibers, microtubules, submicrotubules, unit-tubules, fibrils, monomers, tubulins, etc., all these are nothing but artificially produced microscopic images induced by fixatives of coagulated spindle background substance, the atractoplasm. Of course in future the very terms which have been coined e. g. continuous fibers, microtubules, tubulins, etc. to describe the coagulated features of the atractoplasm will themselves be discarded because, just as with the terms in the coagulated nuclear sap, e. g. network, linins, alveola, etc, they are no longer to be described in present cytology books. The contours in the coagulated atractoplasm may be attributed 1) to differences in fixatives used, with variations in the contours attributed to the different positions of the spindle bodies, and 2) to differences in the resolving powers of the microscope.

Although fixation cytologists including electron microscopists can measure the length and breadth of the coagulated spindle background fibrils or microtubules, they know very little or nothing about the physical properties of microtubules such as their elasticity, viscosity, plasticity, etc. These factors are essential in analysing the mechanism of mitosis. Even if continuous fibers would be replaced with microtubules or tubulins, electron microscopic figures of fixed spindles would scarcely contribute to the elucidation of the various unsolved problems concerning the mitotic spindles mentioned in this paper. Whatever fine structure there may be, the electron microscopists might bear in mind that all such fine structures would not show their dynamic states in living mitotic cells having as they do a high water content.

7. The explanation of the mechanism of mitosis based on the atractoplasm theory

By means of *in vivo* observations and experiments carried out chiefly on the mito-

tic cells of staminal hair cells in *Tradescantia* (Wada 1935–1950), the author elucidated the cause and effect relation in the continuous morphological changes of mitotic figures from prophase to the end of telophase, and proposed a working hypothesis of the mechanism of mitosis (Wada 1950). Since in 1966 the author edited the data and results up to that time and published a monograph "Analysis of Mitosis", he will give here only briefly an outline of the mechanism of mitosis complemented by some of the findings and considerations which have now been confirmed after publication of that monograph.

a. *Role of the spindle membrane and biological significance of the fusiform of the karyokinetic nucleus*

As stated in the morphogenesis of mitotic spindles, the transformation of a spherical prophase nucleus into a fusiform in metaphase has biologically very important significance for the spindle activity in succeeding stages.

According to the general principle of cell physiology, the nuclear membrane makes a barrier restraining the mixing of otherwise freely diffusible intracellular electrolytes (Fujii 1931, Yasui and Fujii 1951, Loewenstein and Kanno 1963). This

Fig. 6. Schematic drawing showing working hypothesis of the mechanism of mitosis based on the attractoplasm theory (see details in the text). a, cell in prophase. The distributions of other than 1) the ions across the nuclear membrane, and also 2) the ATP in the cytoplasm are omitted. b, ions and ATP converge around both spindle poles. c, showing poleward movement of chromosomes by shortening of kinetochore fibers and the appearance of daughter nuclear sap at both polar regions, with no place left for centrioles to play a role in spindle fiber formation (Wada 1970b).

property is of course maintained in the spindle membrane. By a special staining technique Yasui and Fujii (1951) have demonstrated that almost no transfer of electrolytes occurs across the spindle body. The concept of the so-called mitotic apparatus is inadequate for taking electrophysiological activity of spindle bodies into consideration, because the apparatus is explained as appearing after the breakdown of the nuclear membrane.

According to the attractoplasm theory, it is natural to consider that the electrostatic change at the spindle surface membrane plays an important role to promote spindle activity. However, the cell-physiological importance of fusiform transformation of prophase nuclei has been disregarded by cytologists for a long time. It is a well known phenomenon that each pole of the fusiform bodies composes a high

potential region with regard to static electricity, irrespective of whether in living or non-living material. The experiments on the ionic mechanism controlling behavioral responses to mechanical stimulation in *Paramecium* by Naitoh and Eckert (1969) is a noteworthy example of reinvestigation of the electrophysiological phenomena across the spindle membrane with reference to the chromosome movement toward each pole by a shortening of the kinetochore fibers in anaphase.

In prometaphase, as a result of the transformation of a prophase nucleus into a metaphase spindle, it would be natural to assume that the evenly distributed electrolytes across the nuclear membrane in the cytoplasm (Fig. 6a) may move and make a bipolar distribution across the spindle membrane (Fig. 6b). Then it may be electrophysiologically possible to assume that a change of permeability would occur confined to the polar regions of the spindle. Thus, the change of electrochemical gradients and the transfer of metabolites would actively take place at both polar regions across the spindle membrane. The accumulation of adenosine triphosphates and consequently an active energy transfer may become concentrated around both spindle poles (Fig. 6b); and the necessary energy 1) for the spindle activities in anaphase (shortening of kinetochore fibers) and 2) in telophase (organization of daughter nuclei) and reappearance of daughter nuclear sap may be furnished at both spindle regions (Wada 1970b) (Fig. 6c).

b. *Cause and effect relation in the chromosome movement in meta- and anaphase spindles*

According to the attractoplasm theory, it sufficiently strikes one that the morphogenesis of metaphase spindles and their physical properties are ingeniously devised in teleonomy to accomplish without fail the chromosome movement in anaphase by the following *modus operandi*: 1) fusiform formation by the transformation of globular proteins in nuclear sap into fibrous ones, 2) migration of ATP toward the poles of metaphase spindles, 3) increase of electrochemical activity and energy transfer at the polar regions of the spindle, 4) induction of kinetochore fibers along the fibril-tracks toward the poles without fail, and 5) poleward movement of chromosomes by disintegration, in accord with the shortening of kinetochore fibers which occurs limited to both spindle poles (Wada 1950, 1966, 1970b).

This may be the most natural interpretation of the mitotic performance, judged from an evolutionary viewpoint, of the spindles in eukaryota. The breakdown of the nuclear membrane on the employment of centrioles—primordia of apparatus for cell movement (Wada 1969b)—for spindle fiber formation is apparently a misinterpretation based on classical fixation cytology and also based on including electron microscope studies which do not take any consideration of the artifact problems relevant to the continuous morphological changes of the mitotic figures manifested in living cells of high water content.

c. *Morphological changes of mitotic figures which bring about the completion of mitosis*

Various morphological changes taking place at both spindle polar regions in anaphase and telophase may be determined by an energy requirement or by an energy release as follows: 1) the occurrence of disintegration of kinetochore fibers, with a

shortening or pulling of chromosomes toward the poles in anaphase in accordance with this disintegration, 2) the transformation of fibrous proteins into globular ones in telophase, 3) an increase of the daughter nuclear sap by a reformation of the globular proteins in telophase, 4) the reorganization of nucleoli by a gathering of released RNA in telophase, 5) the disintegration of the chromosome matrix and the unravelling of the spiral structures in the daughter chromosome preparatory for entering the metabolic state of the nucleus, and 6) the replication and growth of the daughter nuclear membrane from the polar regions toward the equator of the cell (Wada 1950, 1966). All these phenomena start, at first from both the polar regions of the spindles and some of them continuously spread throughout the whole spindle cavity. The continuous morphological changes of prophase nucleus through meta- and anaphase to the end of telophase were illustrated diagrammatically (Wada 1966 p. 101).

From the results of mitotic cell studies based on *in vivo* observations, it is clear that kinetic centers of the spindle activity in meta- and anaphase lie at each spindle pole enveloped in a spindle membrane, a biological membrane (Wada and Izutsu 1961). Therefore, not only in higher plant cells but also in animal cells it is a self-evident fact that the presence of the nuclear membrane is essential in all the stages of mitosis. In other words, the breakdown of the nuclear membrane described in cytology books is not only an error but also may work to impede the understanding of the mitotic performance in living cells.

d. *Comment on the concept of the so-called mitotic apparatus*

Centrioles generally appear at each spindle pole adsorbing outside of the spindle membrane (Figs. 4a, b) but, though infrequently, they appear at times adsorbing inside of the cell membrane (Fig. 4c). The position of centrioles in mitotic cells is determined physically by various topographic conditions of the cytoplasm: viz. intracellular membranes, vacuoles, inclusions, and also the influence of the polarity of the cell (Nakao *et al.* 1968, Wada 1969b). The so-called mitotic apparatus (Mazia and Dan 1952) very often described in current cytology books is nothing but a revival of the classical cytology of previous days; neither the independence of the nucleus in the metabolic as well as the mitotic state in eukaryota, nor the morphogenesis of the nuclear membrane and the mitotic spindles predicted by gene information had ever been taken into consideration.

Biochemical studies on the isolated mitotic apparatus are now in fashion. Great contributions of biochemistry to biology have been made mostly confined to cells or cell organelles the morphology of which has been concretely established by cytomorphologists, such as the chromosomes, plastids, mitochondria, muscle cells, nervous cells, etc. However on the contrary, the appearance of the mitotic apparatus as due to the breakdown of the nuclear membrane and the isolation of the spindle body away from the apparatus, makes up a mixture of contradictions. Thus it may be the biochemists' illusion of being able to contribute something to biology by treating the so-called mitotic apparatus biochemically without establishing its morphogenesis concretely.

Concluding remarks

Not to be able to point out any development in the descriptions contained in cytology books which the author himself once studied, but rather to have to point out the error of those very descriptions is somewhat unfortunate. However, the currently common idea about the breakdown of the nuclear membrane before spindle formation will not be left without revision for the enlightenment of young biologists studying cytology.

Based on evidence backed up by *in vivo* observations and by improved fixatives and also by taking the evolution of mitotic spindles in eukaryota into consideration, the author has presented a new blue-print for the mechanism of mitosis (Wada 1950, 1966, 1970b). Although this working hypothesis is not the case of a complete theory, yet by removing the misinterpretation of the behavior of the nuclear membrane in mitosis, success has been achieved in elucidating many of the contradictions and unsolved problems in mitosis as described in current cytology books, and also in consolidating the mitotic performance of different types of spindles in higher and lower plant and animal cells under principles common to both.

According to the attractoplasm theory (Fujii 1931), the author has established the nuclear origin of metaphase spindles and confirmed the continuous transformation of the metabolic nucleus into the karyokinetic nucleus viz. the metaphase spindle.

The changes of globular proteins in the prophase nuclear sap into fibrous ones in the metaphase spindle as the basis of the attractoplasm have been proposed from the results of birefringent figures of metaphase spindles in living cells. Accordingly, it will be an essential project for future investigations to confirm this change of proteins quantitatively in living cells and the author is particularly looking forward to the development and the application of dichroic micro spectroscopy.

By means of this biophysical technique, it may be possible quantitatively to confirm the transformation of globular proteins into fibrous ones which occurs in the nuclear cavity. However, to apply this technique to cell biological studies in either *in vivo* or *in vitro* material, various creative technical devices and developments will be required. The beams of incident rays on the material should be nearly 20 μ in diameter and their intensity must be strong enough to pass through the biological material. To arrange fibrous proteins in one common orientation is, however, autonomously arranged in the case of the attractoplasm under the influence of the polarity in the cell.

With regard to the constitution of the attractoplasm at the molecular level, the author has tentatively conjectured various possibilities viz. the folding and unfolding of polypeptides in nuclear sap (Wada 1950, 1966), the reversible polymerization of globular proteins (Wada 1966, 1968a), and the tactoid formation resembling the fusiforms of tobacco mosaic virus. The possibilities of these conjectures have been analytically suggested by some analogous models using dichroic spectroscopy (A. Wada 1972): e. g. such as a) studies on F-actin (a fibrous protein) produced by polymerization of G-actin (a globular protein), b) dichroic spectra of the folding and unfolding of polypeptides (poly- γ -benzyle-L-glutamate), and c) those models of a rod-shaped nucleoprotein aggregate around the RNA core in tobacco mosaic virus.

In the near future, the transformation process of globular proteins into fibrous ones within the framework of the karyokinetic nucleus (viz. metaphase spindle) will be demonstrated biophysically. Any biochemical analysis preconditioning the destruction of cell organelles may contribute very little to elucidate the entity of metaphase spindles biologically.

From the results of mitotic cell studies based on *in vivo* observations, the author has proposed new interpretations of morphogenesis and behavior in seven areas: 1) differences in mitotic figures according to the living state or the fixed state of cells (1969a), 2) behavior of the centriole-aster system (1969b), 3) biological significance of the 9+2 tubloid patterns, the *de novo* appearance of blepharoplast, the disappearance of centrioles in higher plant cells and the formation of flagellum (1970a), 4) the *raison d'être* for the spindle membrane and the atractoplasm being composed of fibrils (1970b), 5) evolutionary aspect of the degeneration of centrioles and the development of cell wall in the plant kingdom (1972a), 6) artifact problems of the mitotic figures under the electron microscope (1972b), and 7) the intrinsic nature of cancer cells and of carcinogenesis as interpreted from the viewpoint in reference to cell biology (1974).

Viewed in this light, it may not be too strong to say that one of the enigmas relative to mitosis is the description of the classic concept of *the breakdown of the nuclear membrane before spindle formation* in current cytology books. Another enigma may be the indifference of animal cytologists toward the presence of the anastatal type of mitosis in the animal kingdom already pointed out in the well known monograph "The Cell in Development and Heredity" edited by E. B. Wilson (1928).

Abstract

This paper is a collective review of the reports (Wada 1935–1974) previously published to corroborate the atractoplasm theory proposed by Fujii (1931) and it also offers a consolidated interpretation of the morphogenesis of mitotic spindles in eukaryota based on comparative studies on protista, metazoa and metaphyta.

From the results of these investigations the author points out that the common idea of the breakdown of the nuclear membrane as one step in the spindle formation in higher plant and animal cells is a gross error. The reasons and the complications by which this error had been overlooked for nearly a century are explained and discussed from various viewpoints, such as the imperfection of observation techniques, the nature of the research material, considerations of evolution relationships, and gene information on the morphogenesis of mitotic spindles in eukaryota.

The author makes clear that many unsolved problems and controversial interpretations of mitosis described in current cytology books are mostly attributed to the misinterpretation of the nuclear membrane in mitosis. He has given answers to these problems.

The morphological independence of mitotic spindles from the cytoplasm, and the transfiguration of metabolic nuclei into karyokinetic ones can be expressed as follows:

The metabolic (resting) nucleus, spherical in form composed of 1) chromonemata,

2) nucleoli, 3) nuclear sap (dispersed globular proteins), and 4) nuclear membrane.

The karyokinetic nucleus (metaphase spindle), spindle-shaped in form composed of 1) chromosomes, 2) kinetochore fibers, 3) atractoplasm (arranged fibrous proteins), and 4) nuclear membrane (a strained state due to enlargement of nuclear volume in the case of higher plant and animal cells).

The morphological continuity of mitotic figures and the cause and effect relations among them are explained in detail from prophase nucleus through metaphase spindle to the appearance of daughter nuclei in telophase, including chromosome movement in anaphase. The biological significance of the spindle-form of karyokinetic nuclei for the mechanism of mitosis is explained.

Acknowledgement

The author is indebted to Drs. Shunpei Inoh, Kosaku Izutsu, and Tsuneyoshi Niitsu for permission to use their photographs in this paper. He wishes to express his sincere thanks to Prof. Akiyoshi Wada, Department of Physics, The University of Tokyo, for valuable suggestions on dichroic spectroscopy and also to Prof. Daniel McCoy, of the Science English Center, Faculty of Science and Engineering, Sophia University, Tokyo, for his kind and continuous help in reading the manuscript.

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