NOTES ON ULTRASTRUCTURE AND SOME PROPERTIES OF TRANSPORT WITHIN THE LIVING MITOTIC SPINDLE

ANDREW BAJER. From the Department of Biology, the University of Oregon, Eugene

INTRODUCTION

The behavior of small granules in living cells has drawn considerable attention during the past few years (1, 11, 12, 15) and has been studied in various organisms. In the present studies their behavior has been visualized in time-lapse films of cells in which it is possible to localize, at the same time, the position of chromosomal fibers. This is only possible in a low percentage of cells observed in vitro with the phase-contrast microscope (2), but in most cells when the Nomarski system is used (3). The granules execute irregular oscillations which are Brownian or saltatory motions, depending on their direction and the time interval between "jumps" in the same direction. According to Taylor (15), it is highly improbable that saltatory and Brownian motions result from the same mechanism. Some general aspects of movements of the saltatory type were analyzed in cells of *Haemanthus* endosperm (1), and the results agree with those later described by Rebhum (11, 12) for eggs of some marine animals and by Taylor (15) for newt cells. No fully satisfactory explanation of the mechanism of saltatory movements has been proposed. However, some evidence exists that the saltatory movements are the result of definite activity of ultrastructural elements (15, 16). It has been suggested that granules located close to fibrillar elements can be transported in certain directions even when the fibrillar elements themselves are not moving in those directions (21).

MATERIALS AND METHODS

The material used was the endosperm of *Haemanthus katherinae* Bak. The methods of handling this material in vitro are described elsewhere (8). Dark-phase contrast and the Nomarski interference contrast system were combined with time-lapse technique which was followed by frame-by-frame analysis. L-W-photo optical data analyzer, Model 224A, (L-W Photo, Inc., Van Nuys, Calif.) was used for analysis of about 200 dividing cells. About 20 cells were selected for more detailed analysis. The behavior of several hundred granules was watched on the screen frame-by-frame. The paths, translations, and other features were measured for more than 50 granules, often for several minutes for each granule. The time interval between measurements was different, and varied from 1–60 sec; for most granules it was 5–20 sec. A detailed report will be published elsewhere.[1]

OBSERVATIONS

The behavior of granules 0.5–0.3 μ in size was analyzed. Such granules are not present or are very scarce inside the prophase nucleus. Numerous granules occur around the prophase nucleus in the clear zone (20), but most of them are eliminated toward the poles during formation of the spindle. Only very few granules are present inside the spindle at the period between prometaphase and anaphase, and are seen in about 10% of the observed cells. Most of the granules are transported toward the poles. The nature and origin of the granules and whether they enter the spindle during prometaphase were not determined. It is also often difficult to determine whether the granule is inside the spindle and its exact position if the granule is within the spindle. Only granules whose location left no doubt were analyzed in detail. This was possible to do mostly in cells studied with the Nomarski system. With phase contrast, selected cells revealed darker zones between kinetochores and the spindle pole which represent chromosomal fibers (2). In such cells it is possible to localize the granules in relation to chromosomal fibers but not so precisely as with the Nomarski system. However, even with the Nomarski system it is not often possible to distinguish chromosomal from continuous fibers.

During prometaphase-metaphase the granules in areas between chromosomal fibers execute Brownian motion, whereas upon entering the areas of chromosomal fibers they are transported toward the poles (Fig. 1) by a movement which has all the features of saltatory motion as characterized by Rebhun (11, 12) and Taylor (15). The change of type of granule movement from Brownian to saltatory was observed several times in the half-spindle. In six cases it was possible to determine the exact moment when the granules enter the area of chromosomal fibers. These were cells

[1] Bajer, A. Data in preparation.

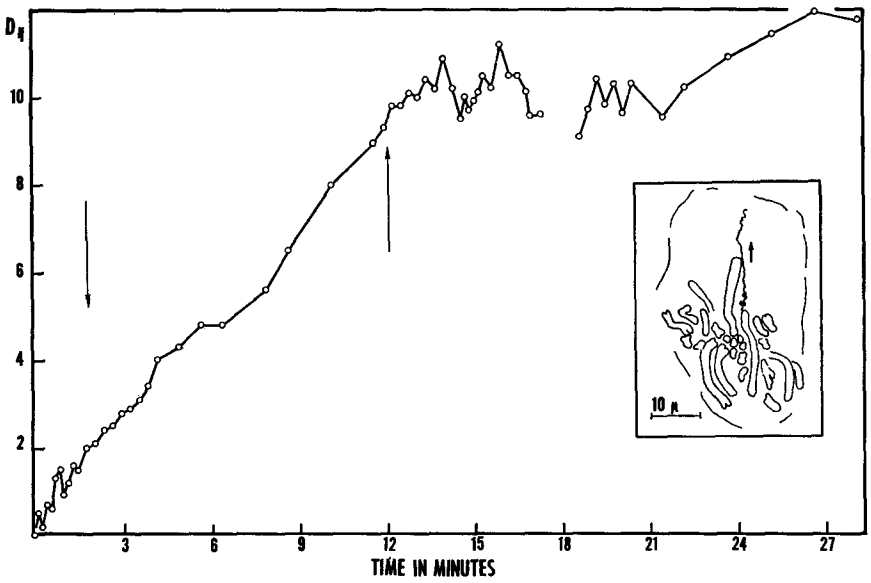

FIGURE 1 Behavior of a granule entering the area of a chromosomal fiber (Nomarski interference contrast system). The distance in microns of the granule from an arbitrarily chosen stationary metaphase plane is plotted against time. During the measurements the plane was considered stationary despite the movement of the kinetochores. The graph does not show the absolute distance travelled by the granule in a given time. Inset, semi-schematic drawing of the cell and the path of the granule. From 2 to 12 min (arrows) the granule is within the area of the chromosomal fibers and travels toward the pole by means of saltatory movement. In the time up to 2 min and after 12 min, the granule executes Brownian motion characterized by both changeable speed and direction, not reflected by the graph. The granule disappears and reappears after about 18 min. The granule leaves the spindle area after about 21 min. The characteristic features of saltatory movement are clearly reflected by the graph: the granule does not move for long distances with uniform speed, and it even stops (time about 6 min).

in which both the shape of the chromosomal fibers and the granules in areas between the fibers were observed. During prometaphase-metaphase, the granules within the areas of the chromosomal fibers move to the spindle pole; the average speed of movement was in the order of the speed anaphase chromosomes, and usually was nearly precisely the same. The speed of movement of the granules decreases as they approach the pole, and at the polar region they again execute Brownian motion. Individual granules vary with respect to their movement toward the pole during prometaphase-metaphase: some of them move with the same speed over long distances; others stop several times during movements. In the area of chromosomal fibers the granules were not observed moving toward the plate. In the very few cases in which the granules moved toward the plate, it was not possible to localize exactly the position of the granules. The total path-length for the granule during saltatory motion is about one-half of that measured during Brownian motion. Both values depend on the frequency of measurements, but the relation does not seem to change. This result is expected for Brownian motion but not for saltatory motion. However, this problem will not be discussed here any further.

If the granules begin their movement toward the pole during metaphase, they continue this movement throughout most of anaphase, traveling with approximately the same speed as the kinetochores, then slowing in later stages of anaphase when the granule reaches the polar region of the spindle. As a result of such behavior the distance between the kinetochore and the granule usually does not change until middle and late anaphase (Fig. 2). The movement during anaphase is more regular than during prometaphase. A few cases were observed in which granules seemed to move slightly faster in early anaphase than the chromosomes. The observations on anaphase are in agreement with results previously reported (9).

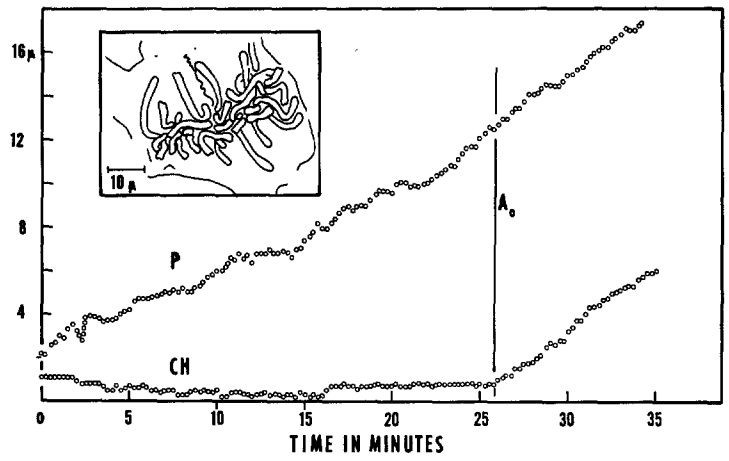

FIGURE 2 Elimination of a granule toward the pole during metaphase and early anaphase. The distances of the granule (*P*) and chromosomes (*CH*) are plotted (from an arbitrarily chosen metaphase plane) against time. Inset: semi-schematic drawing of the cell and the path of the granule. The speed of movement of the granule is nearly the same during both metaphase and early anaphase. The start of anaphase is marked A_0. The speed of granule movement increases slightly during anaphase and is of the same order as the speed of the anaphase chromosome movement. It is not possible to state at present whether the slight increase of speed during anaphase has any significance, but it was observed in several other cells. In most cases, however, the speed of the granule decreases during later stages of anaphase.

DISCUSSION

The behavior of the small granules must be discussed with relation to other movements inside the spindle. It has been established (9, 20) that during the formation of the metaphase plate two kinds of movement take place which are parallel to the long axis of the spindle but in opposite directions: (*a*) kinetochores show a strong tendency to arrange themselves on the equatorial plane, and long chromosome arms are parallel to the long axis of the spindle; (*b*) all bodies, such as akinetic fragments found after irradiation and persistent nucleoli, are transported toward the poles. Therefore, it has been suggested (9) that chromosomal fibers are subjected to the same force during both metaphase and anaphase. It might further be speculated that, as soon as the mechanical connections between two kinetochores disappear at the start of anaphase, kinetochores with chromosomal fibers connected to them are transported in the polar direction as the whole units. Thus, the chromosomal fibers which are composed of many microtubules (*mt*) might act as a kind of support and give guidance during the movement, while the propelling force would be located in the intratubular material. This mechanism might account, at the same time, for the elongation of the spindle. It would also be expected that the transport properties of the spindle during metaphase and anaphase are similar, and that the differences (movements of granules to the pole in metaphase and with chromosomes in anaphase) are different aspects of the same mechanism. The slower movement of granules, when they approach the poles, would occur if the disorganization of microtubules takes place at the polar areas or if their arrangements undergo considerable changes in these regions. Certain aspects of these ideas were presented (2, 9) before any data on the fine structure of the *Haemanthus* spindle were available. Some support for the rather passive role of spindle fibers is supplied by Forer (4) in his experiments on UV irradiation of the spindle in crane-fly meiosis which showed that the irradiated part of the birefringent half-spindle was translocated to the pole.

Suggestions concerning the behavior of microtubules during mitosis are based on the assumption of Östergren (9, 22) that the mechanisms of prometaphase movement and anaphase movement are the same, and that the formation of the metaphase plate is due to a pulling force. Some aspects of the latter part of the hypothesis have been supported on the fine structural level (24).

However, there are also other acceptable explanations of transport properties of the mitotic spindle mentioned above. One explanation would be the oscillation or vibration (21) of microtubules; this might explain equally well all the movements discussed here. There is some evidence that microtubules execute undulating movements, as suggested by Ledbetter and Porter (25). It is hoped that further studies on fine structure combined with observations on the same cells in vitro might supply data in favor of one or another hypothesis.

The work was supported by National Science Foundation grant GB 3335 to the author. The studies with the Nomarski interference contrast system were supported by National Institutes of Health grant GM 08691 to Dr. R. D. Allen, Department of Biological Sciences, State University of New York at Albany, Albany, New York. The author would like also to thank Miss Linda Mackprang for her precise, patient, and exceptionally laborious frame-by-frame analysis of the film.

APPENDIX

SOME ASPECTS OF THE SPINDLE FINE STRUCTURE IN ENDOSPERM CELLS OF *HAEMANTHUS*

For a better understanding of the behavior of granules, some remarks on spindle structure in *Haemanthus* are desirable, especially since several reports have not stated whether bundles of microtubules represent chromosomal fibers (6, 7, 13, 23). However, suggestions that bundles of microtubules in fact form chromosomal fibers have already been given by Ledbetter and Porter (25), and this view has been rather widely accepted (26). Bundles of microtubules forming chromosomal fibers have been clearly shown in prometaphase of sea urchin eggs (24, 27) and in metaphase of *Haemanthus* (5). Rather conflicting evidence has been presented by Behnke and Forer (17), who did not find that microtubules are arranged in bundles. Those authors suggest that birefringent areas do not correspond to chromosomal fibers. However, they did not indicate clearly what stages were studied; it has been found in *Haemanthus* that the arrangement of microtubules changes considerably during formation of the metaphase plate.[2] The connection of bundles of microtubules with kinetochores or kinetochore-like structures seems to be of rather general occurrence, for it has been reported in material so different as amebas (23), sea urchin eggs (23, 27), and animal tissue cultures cells, and in both mitosis and meiosis (26). In *Haemanthus* endosperm cells, the spindle in late prometaphase, metaphase, and part of anaphase seem to be composed of bundles of microtubules connected to kinetochores and bundles connecting two polar areas, i.e., continuous fibers. There seems to be no doubt that each bundle connected with kinetochore contains 50–100 microtubules (5), or even more[2] represents a chromosomal fiber (Figs. 3, 4).

In the equatorial area of the spindle spaces exist between bundles where microtubules are very scarce or are not detected. Since the structure of the spindle changes between early and late prometaphase, it is rather important that the same whole intact cell be observed with the light microscope before sectioning for electron microscopy in order to obtain the exact identification of stages. This is possible for *Haemanthus* endosperm for which a technique has been developed[2] to permit detailed observations of the same cell in vitro and subsequently in thin sections with the electron microscope. The location of the same parts of the cell, e.g. kinetochores, with the light and electron microscopes presents no difficulty, and fixing of cells can be done at the desired stage of mitosis. Not enough data are available at present to permit a description of the formation and disintegration of the chromosomal fibers at the light and electron microscope levels. However, there is some evidence that intermingling of microtubules from different chromosomal and continuous fibers takes place during the formation of the metaphase plate and is very pronounced during anaphase. It has not been determined whether continuous fibers are all bundles of microtubules which did not get connected with kinetochores. In some cases microtubules seem to be connected with or to penetrate into parts of chromosomes other than kinetochores in different stages of mitosis.[2] The role of such microtubules is not yet clear, but similar observations have been reported for other material (7, 17). Cases are known, however, in which such connections were not detected (10, 15, 23). The Nomarski interference contrast system (2)[1] clearly shows the chromosomal fibers in the living cells (Fig. 3), permitting changes to be followed between prometaphase and telophase. The shape of chromosomal fibers as seen with the

[2] Bajer, A. Fine structure studies on mitosis in endosperm. Data in preparation.

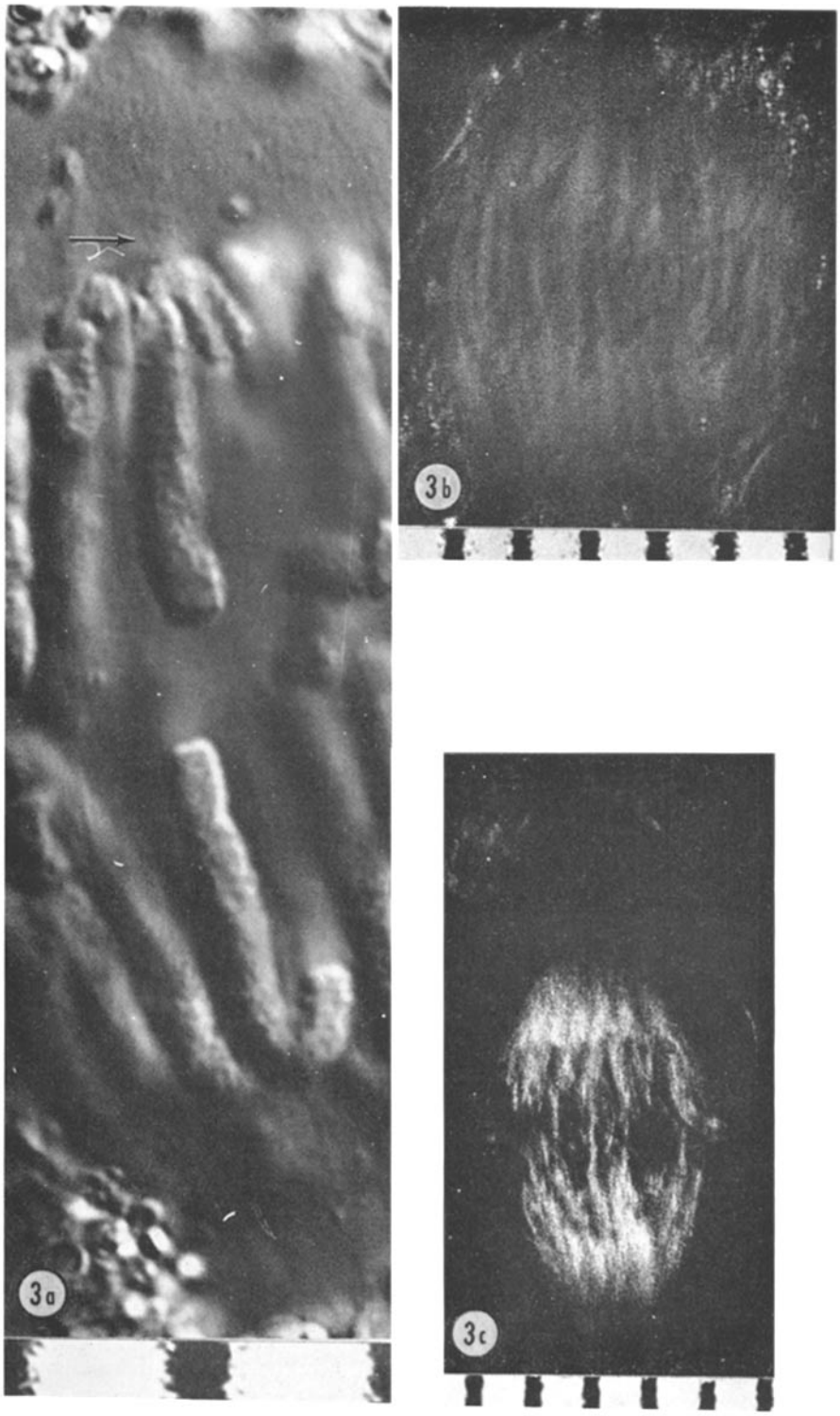

FIGURE 3 Chromosomal fibers in the light microscope. (*a*) Nomarski interference contrast system. Living cell. Chromosomal fibers attached to the kinetochore and diverging towards the poles are seen (arrow). Other fibers at different focal planes are not seen. Some granules between chromosomal fibers are seen. Y-shaped line under the arrow is dust. *b–c*. Polarized light. (*b*) Living cell in middle anaphase, slightly earlier stage than in (*a*). Some granules between chromosomal fibers are seen. (*c*) Acetic alcohol-fixed cell. In both cases the chromosomal fibers converge towards the poles. *a–c* 10 μ scale on all figures. (*a*) × 2600; (*b*) × 1000; (*c*) × 800.

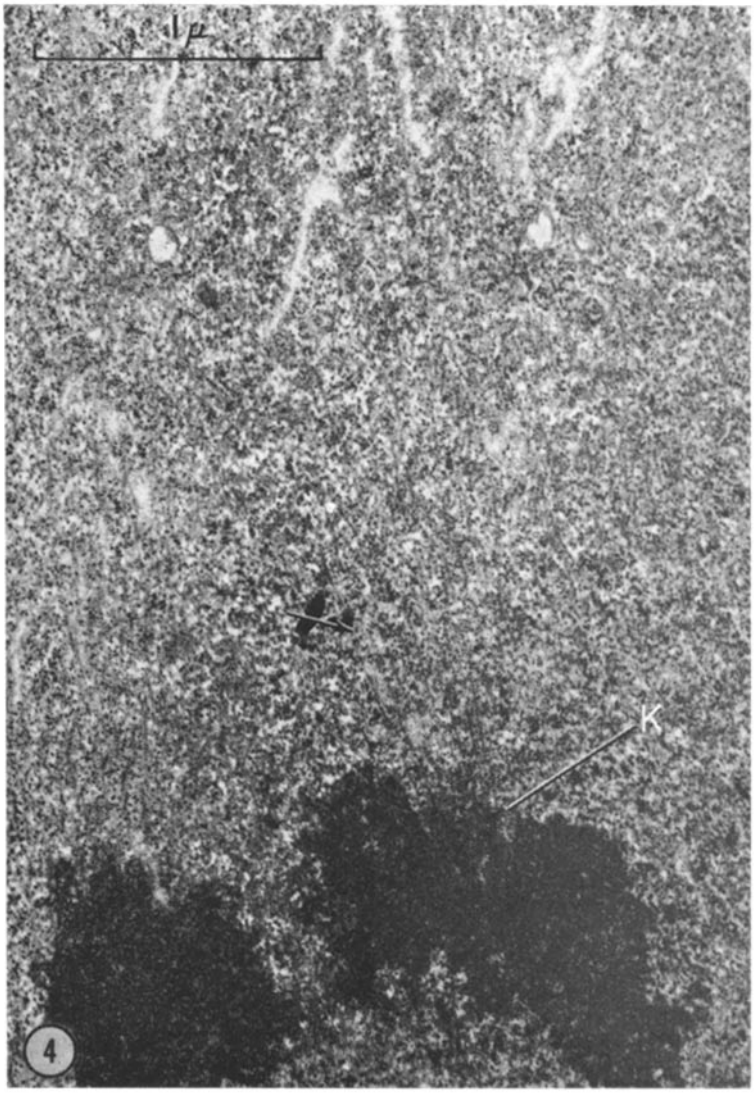

FIGURE 4 Chromosomal fibers in the electron microscope. Early anaphase cell fixed in glutaraldehyde (18% glutaraldehyde in phosphate buffer, pH 6.9) postfixed in osmium tetroxide (1.0% OsO in phosphate buffer, pH 6.9) and stained with lead citrate and uranyl acetate. Microtubules forming chromosomal fibers (arrow) attached to kinetochore (*K*) and diverging towards the pole are intermingling with the bundle of microtubules to the right. The kinetochore of the latter bundle is not seen in this micrograph, but is found in the other serial sections. As estimated from other sections, less than 100 microtubules are connected with the kinetochore (*K*). This cell was fixed in early anaphase when the distance between kinetochores was about 7 μ. The black areas through which the arrow passes are dirt. (Cell 81/66). × 38,000.

Nomarski system is similar to the shape of the whole bundle of microtubules connected with the kinetochore as seen with the electron microscope, although single microtubules obviously are not resolved with the light microscope (Fig. 3). Individual chromosomal fibers seen with the Nomarski system and bundles of microtubules seen with the electron microscope (Fig. 4) diverge during metaphase as they approach the poles and intermingle with other bundles between kinetochores

and polar region of the spindle. The shape of the chromosomal fibers as seen with both systems is not the same as that observed with polarized light. With polarized light, *Haemanthus* chromosomal fibers (see Fig. 3 and the micrographs of *Haemanthus* endosperm in references 2, 5, 18, 19) have a tendency to taper in the polar areas of the spindle (19, discussion), and intermingling is not clearly seen.

SUMMARY

Time-lapse filming technique was used to study the behavior, between prometaphase and late anaphase, of small granules in precisely localized areas within the mitotic spindle of endosperm cells of *Haemanthus katherinae* Bak. During prometaphase-metaphase, granules found between chromosomal fibers execute Brownian motion, whereas those entering the area occupied by the chromosomal fibers, which correspond to dense accumulations of microtubules, are, as a rule, transported toward the polar region. During anaphase the small granules move toward the pole at the same speed as the anaphase chromosomes. Some evidence is presented to indicate that during anaphase a considerable part of the chromosomal fiber close to the kinetochore is translocated toward the pole.

Received for publication 1 November 1966.

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