

THE FINE STRUCTURE OF THE MID-BODY OF THE RAT ERYTHROBLAST

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ABSTRACT

The development of the mid-body has been studied in mitotic erythroblasts of the rat bone marrow by means of thin sections examined with the electron microscope. A differentiated region on the continuous spindle fibers, consisting of a localized increase in density, is observed at the equatorial plane. The mid-body seems to develop by the aggregation of such denser lengths of spindle fiber. Its appearance precedes that of the cleavage furrow. A plate-like arrangement of fibrillary material lies transversely across the telophase intercellular bridge. Later, this material becomes amorphous and assumes the form of a dense ring closely applied to a ridge in the plasma membrane encircling the middle of the bridge. Although the mid-body forms in association with the spindle fibers, it is a structurally distinct part, and the changes which it undergoes are not shared by the rest of the bundle of continuous fibers.

INTRODUCTION

From studies of the spindle of dividing animal cells made by polarized light microscopy (14, 15) and by electron microscopy (2), as well as from certain classical observations reviewed by Schrader (21), the existence of oriented material, commonly called the "spindle fibers," has been firmly established, at least for many types of cells. According to Bernhard and de Harven (2), the fibers of the various parts of the spindle (aster, chromosomal fibers, and continuous fibers) have an identical fine structure in mammalian cells.

The central spindle, containing the continuous fibers, appears to give rise in late telophase to a small basophilic structure, the mid-body, lying in a bridge connecting the daughter cells (10). This structure is called, variously, the mid-body, intermediate body, Zwischenkörper, or Flemming body. There is great variation in the size, position, and duration of existence of the mid-body from one type of cell to another (11). It is absent from plant cells and a few animal cells. In general it

persists for a considerable time after the other events of cytokinesis are completed (13).

The appearance of the mid-body in electron micrographs has been described in mammalian secondary spermatocytes (5-8) and in enidoblasts of *Hydra* (9), in both of which it persists well into interphase.

The erythroblasts of bone marrow offer favorable material for the study of the mid-body because of the high incidence of mitotic cells and the small size of the cells. These factors make it possible to observe a large number of cells showing various stages of its development. We have attempted to trace the formation of the mid-body from the material of the spindle and to observe the subsequent changes in its morphology.

METHODS

For the purpose of inducing hyperplasia of the bone marrow, rats were bled from the tail vein at intervals of several days beginning a week or more before

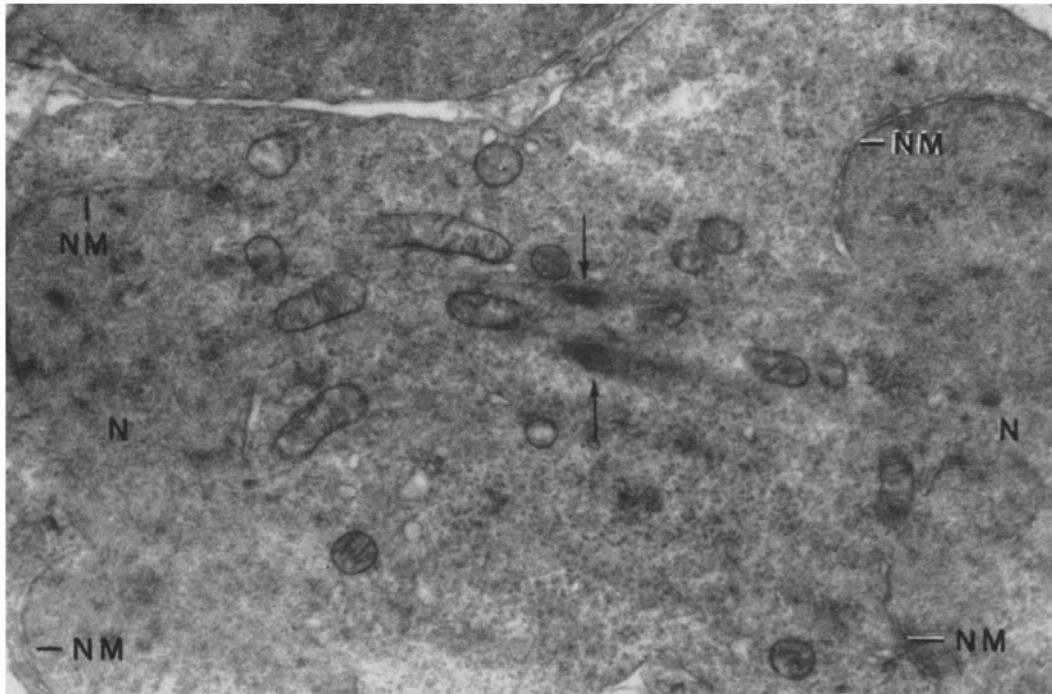


FIGURE 1

Early telophase, showing nuclei of daughter cells with formation of nuclear membrane (*NM*) completed only on the polar surfaces. There is a slight equatorial narrowing of the cell. At the equatorial plane are seen two dense structures (arrows) from which fibrillary material radiates toward the daughter nuclei (*N*). The dense structures represent differentiated parts of the spindle. These parts seem to have become prominent by the massing together on the equatorial plane of short lengths of dense, fibrillary material associated with or part of the continuous spindle fibers. $\times 15,000$.

sacrifice. Bone marrow of the femoral shaft was fixed for 1 hour in cold 1 per cent buffered osmium tetroxide at pH 7.3, treated for 1 hour with cold 1 per cent aqueous uranyl acetate, and embedded in Epon (19) or Vestopal W (17). Thin sections, mounted on uncoated grids, were examined in an RCA EMU-3D electron microscope.

OBSERVATIONS

In the metaphase cell, spindle fibers are seen which appear to extend from centrosphere to kinetochore, and others appear to pass through the metaphase plate but show no kinetochore attachment. The latter are probably the continuous fibers, but in our preparations of erythroblasts their identity becomes definite only with the onset of telophase, when a fibrillary material is seen between the separating chromosome masses (Fig. 1). The striking feature of the continuous

spindle fibers is that, even in images of very early telophase, the fibrillary material shows short lengths of increased density in a position which is halfway between the separating chromosomes. Further work will be necessary to trace the origin of these areas to the earlier stages, although they have been seen in early anaphase in other kinds of cells (4). In a single section of the erythroblasts one or two tiny points of increased density are seen spread out over the axial one-half or more of the equatorial plane. Later in telophase the region of the equatorial plane which they occupy is reduced. The points of density are then much larger, apparently because they become fused together. This condition of the spindle is seen before the cleavage furrow has developed beyond the stage of slight equatorial indentation (Fig. 1).

Still later in telophase the cleavage furrow extends from the narrow intercellular bridge to a point at the periphery of the cell corresponding to the slight equatorial indentation. The development of the furrow is discussed in a subsequent publication (4). Occupying most of the telophase bridge, when it first forms, is a plate of dense, fibrillary material (Fig. 2) which corresponds to the dense part of the spindle described earlier. The plate in the telophase bridge constitutes the mid-body.

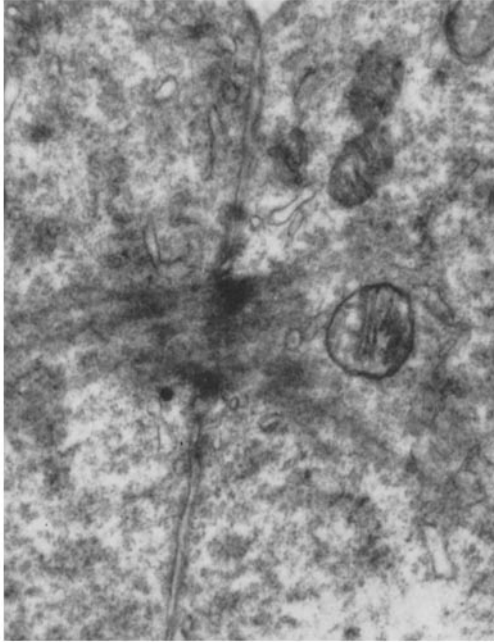


FIGURE 2
Mid-body and the fibers of the spindle. The very short bridge connecting the daughter cells suggests that the cleavage furrow had developed only a short time before fixation. $\times 24,000$.

In the erythroblast the mid-body lies eccentrically, so that in sections cut longitudinally the spindle fibers may appear to arch over the nuclei and run for a distance between the nucleus and plasma membrane (Fig. 3). Other sections, also cutting the telophase bridge longitudinally but at 90° to the first, show spindle fibers apparently fanning out toward the nuclear membrane.

The telophase bridge appears very short in some sections (Fig. 2, for example) and longer in others (up to 6μ). The very short bridges are sometimes found even before the nuclear mem-

brane is completely re-formed, and it seems probable that the longer bridges, in general, represent a later stage of development than the shorter ones. It must be admitted, however, that we have been unable to discover any criterion, other than the length of the bridge, which would permit us to arrange the various images of the mid-body in a correct time sequence. Therefore, in the description which follows we have created a sequence of developmental stages which further work may

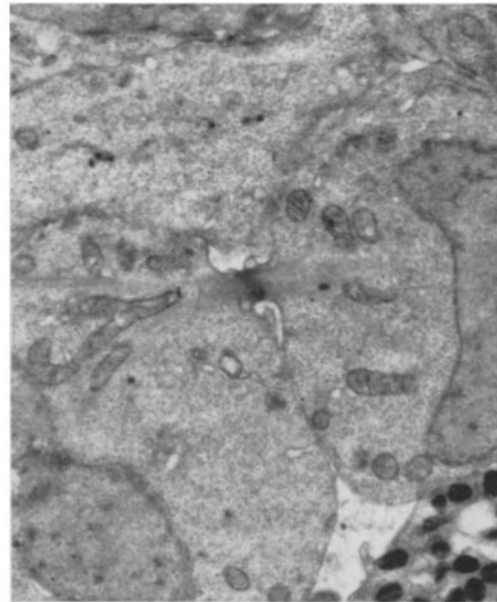


FIGURE 3
Eccentric position of the spindle and mid-body. The telophase bridge is short, and, although the nuclear membranes are now fully developed, the disc-like shape of the nuclei indicates that this is a rather early stage in the existence of the telophase bridge. $\times 8,000$.

show to be incorrect, but which appears to us, at this time, to be the most probable one.

In short bridges, as stated above, the mid-body consists of fibrillary material, the units being arranged so as to coincide and form a disc or plate (Fig. 4) whose length along the long axis of the bridge measures from 100 to 300μ .

In the early stages the disc-like mid-body extends across most of the telophase bridge. Images have been obtained which can be arranged in a sequence suggesting that the central part of the disc later becomes devoid of the dense material.

The solid disc thus becomes a ring. At the same time the dense material loses its fibrillary character and becomes amorphous. An early stage is illustrated in Fig. 5. Here a few axially located filaments are still present. Later stages are shown in Figs. 6 and 7. The plasma membrane about the middle of the bridge becomes progressively more bulging, and dense amorphous material fills this ring-shaped ridge. The appearance of a bulge of the plasma membrane at the mid-body is partly due to the considerable decrease in the diameter of the rest of the telophase bridge. In

are sparse and inadequate, and a conclusion about the means of final separation of the cells is not yet warranted.

DISCUSSION

When the cell flattens in anaphase, the spindle assumes a cylindrical shape (14, 18). The bundle of continuous fibers at this stage comprises the "Stemmkörper" of Bělár (1), and as it undergoes elongation it is thought to push apart the poles of the cell, perhaps with the assistance of the contracting fibers of the aster (3, 16, 20). The chromo-

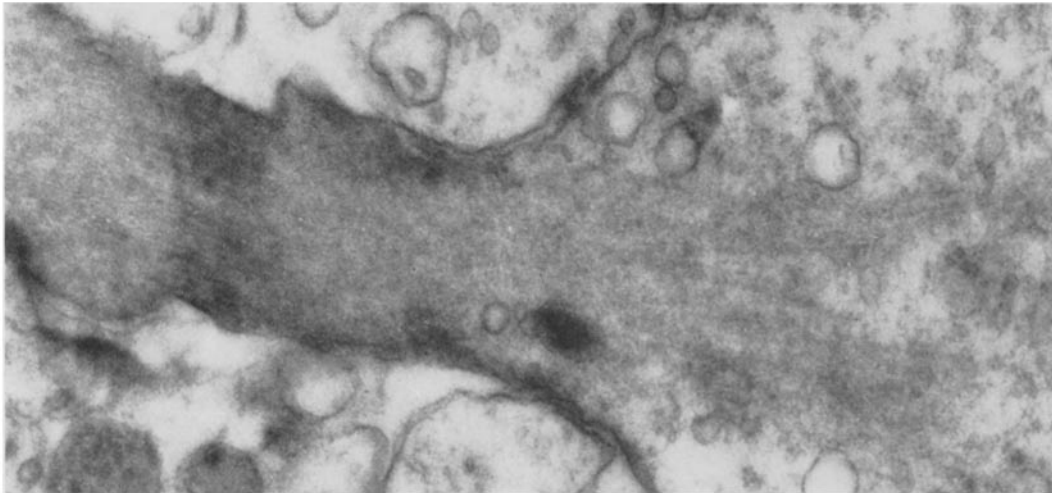


FIGURE 4

Telophase bridge of moderate length, showing plate-like mid-body composed of dense fibrils. The fibrillary material of the spindle extends into the adjoining cytoplasm. $\times 42,000$.

images which are interpreted as still later stages, the dense material appears to be hollowed out as it is closely applied to the encircling ridge of membrane (Fig. 7). Even in images of such a late stage, the fibrillary material of the spindle is usually seen running from the bridge into the adjoining cytoplasm of the daughter cells (Figs. 5 to 7).

The final process of the rupture of the bridge is not conveniently studied in thin sections because of the difficulty of distinguishing truly parted bridges from those sectioned obliquely. We have observed, occasionally, a row of tiny vesicles or pieces of membrane running transversely across the bridge (Fig. 8), suggesting the *de novo* formation of a membranous partition to one side of the middle. However, our observations on this point

somal spindle fibers are then in a state of maximal shortening (22). It is at this stage that we have observed the first evidence of the densities associated with the spindle fibers. At the present time it is futile to speculate on the significance of these points of density, although the possibility should perhaps be considered that they may represent the sites of active growth of the spindle during the period of separation of the chromosomes.

It is significant, at any rate, that before the cleavage furrow appears, points of differentiation are seen on the continuous fibers. According to Flemming (10), the mid-body develops by the fusion of a number of fine beads on the equatorial plane. Our interpretation would agree with this early observation. The more usual view is that the mid-body results from the compression of

the spindle brought about by the advancing margins of the cleavage furrow (11, 12, 20, and see 9 for review). In the erythroblast it is not possible that the mid-body is formed by the compression of an ingrowing furrow, for it has already become a recognizable structure before the furrow develops. Moreover, as will be shown in a subsequent publication, it is likely that the furrow does not grow toward the axis of the cell, but begins at the mid-body and grows out (4).

The sequence of changes in the mid-body, as we have described them, is consistent with the

The erythroblast mid-body of the early stages, forming a transverse plate, resembles that described for cnidoblasts of *Hydra* by Fawcett, Ito, and Slautterback (9). In later stages the mid-body of these cells also showed an encircling ridge of plasma membrane at the mid-point of the bridge, although no significant amount of dense material was found on this part of the membrane. On the other hand, in the secondary spermatocyte bridges described by Fawcett and Burgos (5, 8) a ring of dense material encircled the bridge but the plasma membrane did not show a ridge. In the



FIGURE 5

Early stage in the margination of the dense material of the mid-body. Some of the dense material is fibrillary, but most of it is amorphous and forms a ring against the plasma membrane. $\times 24,000$.

observations of Hughes and Swann (14), who studied birefringence in the spindle of chick fibroblasts. They observed intense birefringence in the spindle of late telophase which gradually decreased as the cells separated. At its height, the birefringence was more intense than that of the whole metaphase spindle. Thus, this period may correspond to the plate-like form of the mid-body, and the gradual decrease may signify the loss of oriented material as the later type of amorphous mid-body develops.

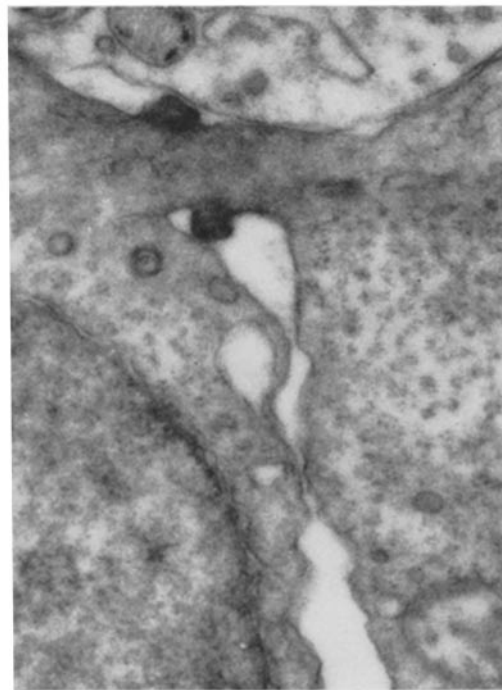


FIGURE 6

The amorphous dense material of the mid-body lies in a bulging ring around the telophase bridge. $\times 42,000$.

spermatocytes longitudinally oriented spindle fiber remnants were not seen, but they were present in the *Hydra* cells.

The observations of Fawcett's group, taken with our own, suggest that the material of the mid-body is distinct from that of the rest of the spindle. We have observed that when the fibrillary material of the early mid-body changes to amorphous material the rest of the spindle is still fibrillary. As stated above, the mid-body of spermatocytes

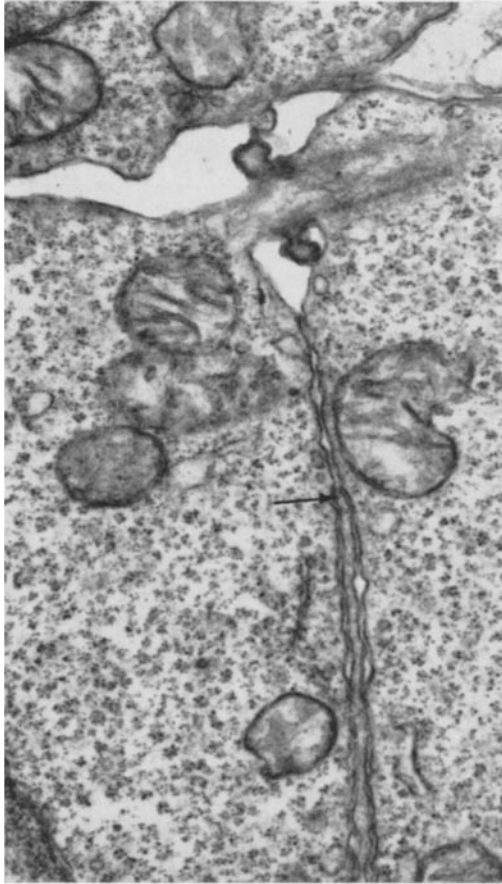


FIGURE 7

The bulging ring of the mid-body consists of a layer of dense material applied to the plasma membrane. Fibrillary material of the spindle runs into the cytoplasm of the daughter cell on the right. The telophase bridge lies eccentrically, and a long process of an adjacent cell extends into the cleavage furrow (arrow at tip of the process). $\times 33,000$.

can be observed even when spindle fibers are not seen. Perhaps this observation reflects only the transient nature of the spindle fibers in these cells or their instability in the fixing fluid, but it does, nevertheless, point to some essential difference between these two structures.

Thus, although the mid-body develops in association with the continuous spindle fibers, its formation is not the result of the fibers' being passively pressed together. From the standpoint of its morphology in the early telophase spindle and from the later changes which it undergoes,

which are different from those seen in the rest of the spindle, the mid-body may be considered a cytoplasmic organelle with distinctive morphological, and perhaps chemical, characteristics. In a later publication (4) the functional significance of the mid-body will be discussed in relation to the development of the cleavage furrow.

This work was supported by a grant from the Medical Research Council of Canada.

The electron microscope was supplied by the National Cancer Institute of Canada.

The authors are grateful to Messrs. William Daniels and Charles Jarvis for technical assistance.

Received for publication, November 2, 1961.

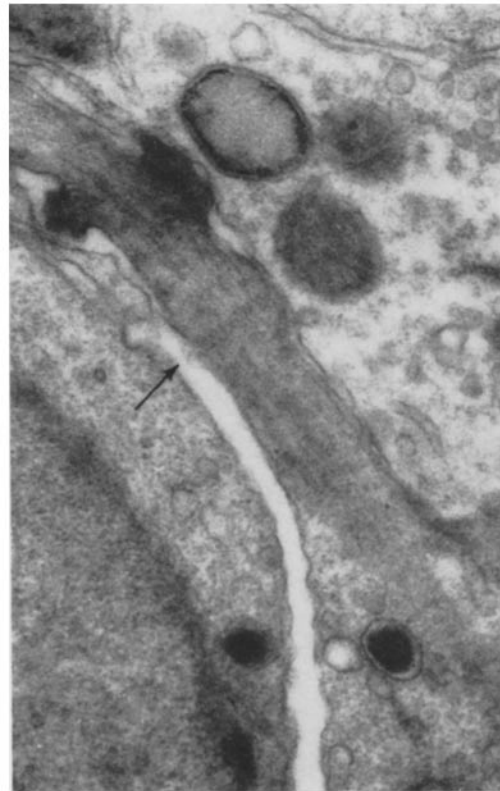


FIGURE 8

A long telophase bridge, showing the ring form of the mid-body and also a length of the bridge which is slightly wider than the rest. This part is traversed by a row of tiny vesicles (plane of the arrow). $\times 37,000$.

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