SOME OBSERVATIONS CONCERNING METAKINESIS IN SEA URCHIN EGGS

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One of the more perplexing aspects of mitosis has been the nature of the mechanism whereby the chromosomes are moved into their metaphase configuration. Early theories of chromosome movement which involved the spindle or chromosomal fibers later gave way to theories proposing alternate mechanisms, as spindle fibers came to be regarded as artifacts of fixation. In recent years, however, evidence for the actual existence of spindle fibers has accumulated, notably Inoué's studies showing the birefringence of the spindle in living material (Inoué, 1953). Recently improved electron microscope techniques have now revealed great numbers of fine filaments having a tubular appearance and a diameter of approximately 15 to 20 mμ in osmium tetroxide–fixed material (Roth *et al.*, 1960; Roth and Daniels, 1962; Harris, 1962; and others). The present paper describes the fine structure of the mitotic apparatus at prometaphase of the first cleavage division, showing the early attachment of these filaments to the chromosomes and their insertion into the centrosphere region at the poles.

MATERIALS AND METHODS

Eggs and sperm of the sea urchin *Strongylocentrotus purpuratus* were obtained by injecting the sea urchins with 0.5 M KCl, allowing the eggs to shed into a beaker of sea water, and collecting the sperm "dry" in Syracuse dishes. Following fertilization, the eggs were treated for about 15 minutes with mercaptoethylgluconamide (0.2 gm per 200 ml of egg suspension) to prevent the hardening of the fertilization membranes, which were then stripped off by passing the eggs through fine-mesh silk screen. This treatment, while leaving the hyaline layer and other surface structures unaltered, facilitates fixation and penetration of embedding medium during later operations. Following the stripping, the eggs were washed and returned to normal sea water for development at 15°C with constant stirring.

The developmental stages were monitored with the light microscope. At the time of beginning breakdown of the nuclear membrane, in this case about 75 minutes after fertilization, the eggs were collected and fixed for 3 hours in 1 per cent osmium tetroxide in 0.5 M sodium acetate at pH 6.1. The function of the sodium acetate was to bring the osmolarity of the fixative close to that of sea water, while at the same time serving as a weak buffer. Subsequent serial sectioning indicated that the division stages in this sample ranged from cells in which the nuclear membrane had not yet broken down, to prometaphase stages where the nuclear membrane had almost entirely disappeared. None were observed in metaphase or later stages. The fixed eggs were dehydrated in graded ethanols and embedded in Epon epoxy resin. Sections were cut with a Porter-Blum microtome, stained with lead hydroxide, and observed with an RCA-3F electron microscope.

OBSERVATIONS

In cells where the nuclear membrane has not yet broken down, no spindle filaments can be seen either within the nucleus or in any way related to the condensing chromosomes. However, immediately following the first visible breach in the nuclear membrane, numerous spindle filaments can be seen within the nuclear region, and these very early become attached to the kinetochores of the chromosomes. These filaments, having the appearance of tubes with a diameter of 15 to 20 mμ, become aligned in massive array to form the central spindle, along with the continuous filaments

that extend from pole to pole. The chromosomal filaments, as described earlier (Harris, 1962), appear to attach directly to a dense kinetochore region at the surface of the chromosome (Fig. 1). At this stage there are two of these dense attachment regions, each oriented toward its respective pole. The filaments retain their parallel orientation until they reach the centrosphere region of the aster, where they suddenly lose this orientation and can be seen as short segments or in cross-section. Such a configuration could be interpreted as either broken or folded. This abrupt change in structural characteristics between the centrosphere and the outer region of the aster and spindle is best seen at low power in very thick sections, and is especially striking in preparations of whole isolated mitotic apparatus. Fig. 2 shows a thick section of one of the asters of a mitotic figure isolated in digitonin. At this magnification the aster fibers appear to end at the periphery of the centrosphere. Fig. 3 shows the polar connection of the same filaments shown in Fig. 1, covering an area equivalent to that indicated in Fig. 2. While the boundary here at higher magnification is not so sharply defined, one can see, however, the difference in orientation between the spindle filaments and the segments of filaments visible in the centrosphere. This centrosphere region, with its disarrayed filaments, grows steadily in size from very early prophase until it reaches rather huge proportions in late anaphase or telophase, a process most striking in large invertebrate eggs, as noted by Wilson (1924, p. 676) and in studies of isolated mitotic apparatus carried out in the laboratory of Dr. Daniel Mazia (unpublished observations).

No direct connection of filaments to the centrioles has been observed, either in the first division cells of this study, or in other studies of later embryos where the aster is either much reduced or not present at all. The filaments either disappear in the immediate vicinity of the centrioles, or change their orientation with respect to the spindle axis.

The data presented here confirm at the fine structural level the light microscopic observations on fixed material and polarization studies on living cells, and support the idea that chromosomal fibers are instrumental in moving the chromosomes to the metaphase plate. If the fine structure of the centrosphere and its continuous growth from prophase to telophase have any meaning in terms of possible pulling forces on the chromosomal filaments, then the data presented here would also support the idea of a continuous force which moves the chromosomes to the equator and does not require a reversal of direction at metaphase to move the chromosomes to the poles.

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FIGURE 1 Chromosomes (*CH*) and spindle filaments at prometaphase, showing the dense attachment regions on the surface of the chromosome. × 42,000.

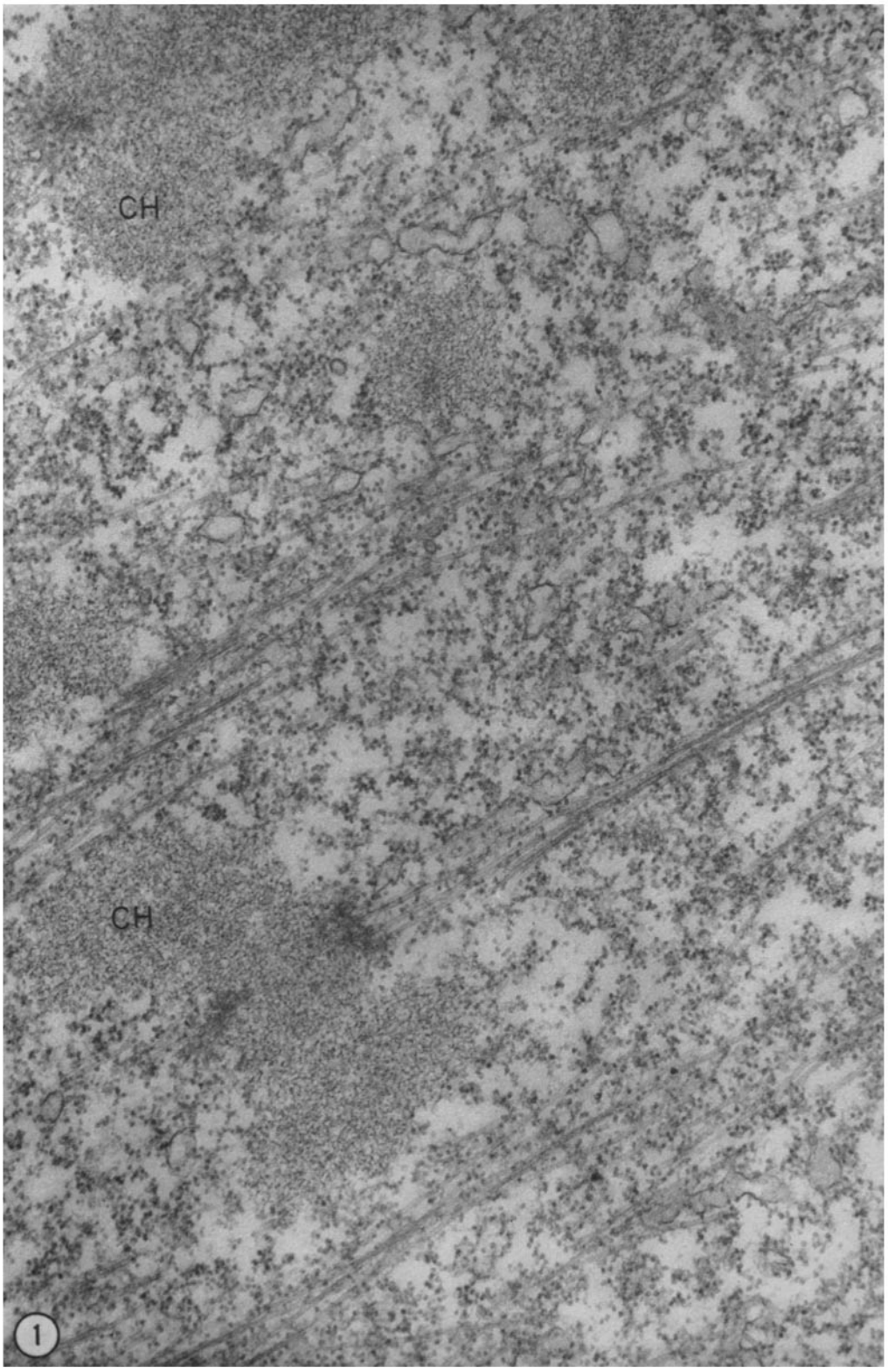
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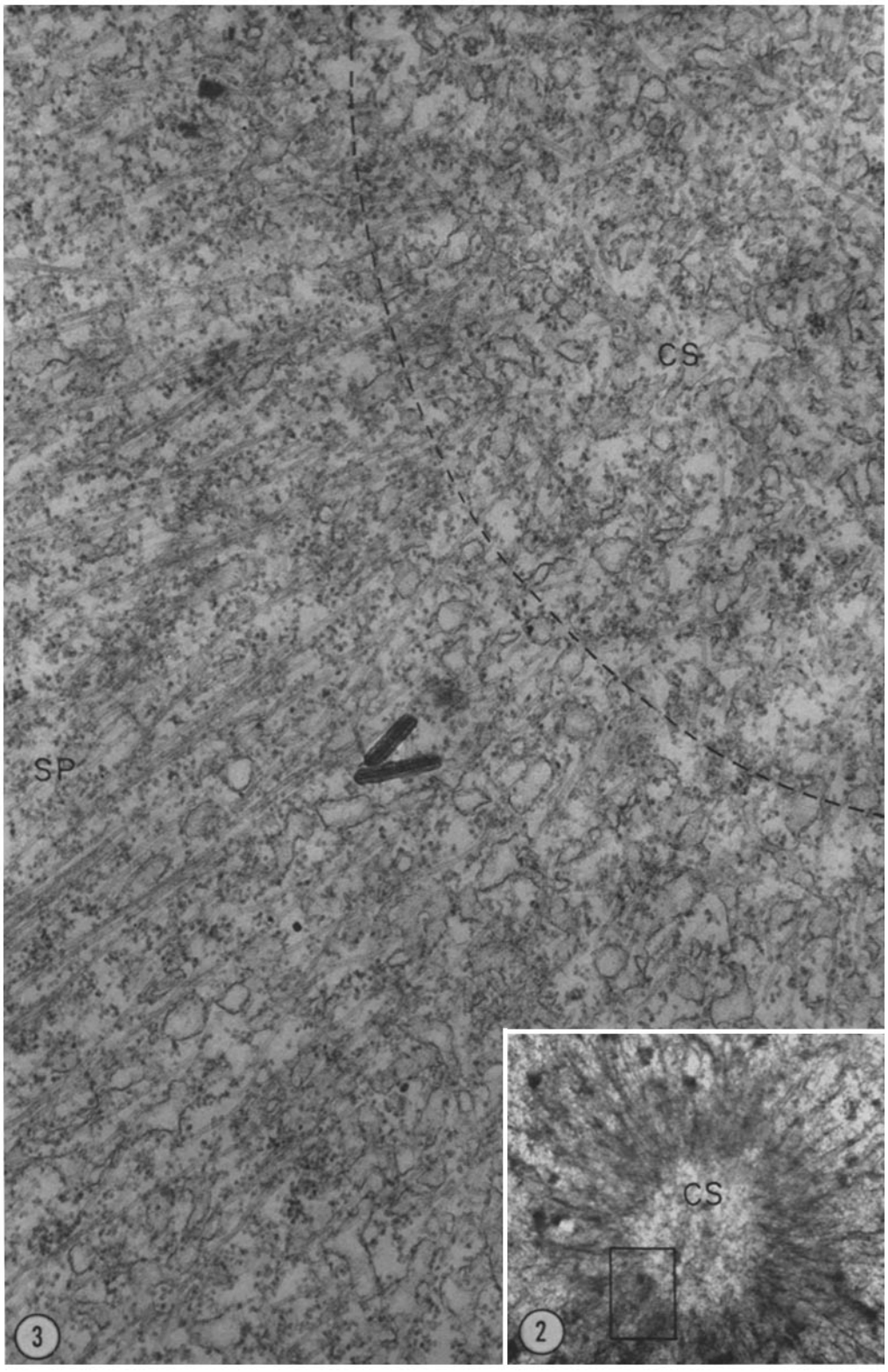
FIGURE 2 Low power electron micrograph showing a thick section of one of the asters of a mitotic figure isolated with digitonin. Here the fibers or aggregations of filaments appear to end at the periphery of the centrosphere (*CS*). The area outlined is equivalent to that shown at higher magnification in Fig. 3.

FIGURE 3 Polar end of the same spindle shown in Fig. 1. Oriented spindle filaments (*SP*) and filaments from the outer region of the aster can be seen merging with the highly disoriented region within the centrosphere (*CS*, outlined by broken line). Black object in center of field is dirt. × 42,000.


CS
SP
3
CS
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