DEMONSTRATION OF A COLCEMID-SENSITIVE ATTRACTIVE FORCE ACTING BETWEEN THE NUCLEUS AND A CENTER

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INTRODUCTION

An association between the nucleus and the cell center[1] has been recognized since centers were first described by Boveri. The basis of the association is unclear. In electron micrographs centrioles are not usually found in contact with the nuclear membrane, and the intervening material is not uniquely structured although microtubules are frequently seen (4, 5).

In this paper, I have considered the possibility that the connection between the nucleus and the center is maintained by an aster-associated motile system. Astral ray–associated motility has been recognized for almost one hundred years (1) but, since the nucleus is usually found near the center, nuclear movement is not readily seen. Movement of the female pronucleus is a probable exception.

Astral ray- and microtubule-associated motility is often Colcemid sensitive (6, 7). If the interaction between the nucleus and the center is also Colce-

[1] Wilson (1, p. 119), Mazia (2, p. 117), and Went (3) have discussed some of the considerations and difficulties in defining a center (Wilson's central body) precisely. Despite studies giving better information about astral rays, centrioles, and satellites, Wilson's "vague" definition which does not require a centriole still seems pertinent.

mid sensitive, then it may be possible to establish conditions such that the aster and the nucleus can be separated. Continued application of Colcemid would then greatly reduce the size of the aster, producing a stable separation of the nucleus and the center. Finally, removing the Colcemid block would lead to recovery of the astral rays and to movement of the nucleus to the center.

MATERIALS AND METHODS

Eggs and sperm from the sea urchins *Lytechinus variegatus* and *Lytechinus pictus* were used for these observations. Spawning was induced by injecting 0.5 M KCl. Artificial sea water prepared according to the formulation of the Marine Biological Laboratory (Woods Hole, Mass.), was used throughout. The experiments were carried out in Philadelphia at room temperature (about 22°C).

Fertilized eggs were placed in 1×10^{-6} M Colcemid (N-methyl N-desacetyl colchicine) for 10 min at that time when 50% of the eggs had cleaved. After 10 min the eggs were centrifuged in Colcemid against a 1 M sucrose cushion at forces up to 30,000 *g* for 3 min. After centrifugation the cells were mounted in artificial sea water without Colcemid for observation. Minor modifications in this procedure were commonly made in order to increase the frequency of separation. The effect of Colcemid was reversed photochemically by irradiating at 366 nm as described previously (8).

RESULTS

In cells centrifuged in Colcemid the nuclei were found at the centripetal pole where they were frequently overlaid with negatively birefringent material, possibly endoplasmic reticulum or annulate lamellae. Astral ray birefringence was not usually seen nor did it reappear without irradiation. In a given preparation a cell was selected for orientation of the furrow and of the nucleus, and then was irradiated with 366 nm light to see where the asters were. The frequency with which nuclei separated from their centers was variable and depended partially on the eggs used. It was probably never above 50% and often seemed near 0.[2]

Immediately after irradiation, a center was apparent as a small radially birefringent region which continued to increase in diameter and in birefringence over the next few minutes. When the center was separated from the nucleus, there was an interval of up to several minutes before the nucleus moved directly towards the nearest center at speeds estimated by eye to be 0.3 μ/sec. Clearly visible astral birefringence did not extend to the nucleus when it started to move. Elongation of the nucleus in the direction of movement was common.

Two centers with varied separation were usually present after irradiation since most experiments were done in a period beginning about 15 min before prometaphase. This provided a situation in which a loose nucleus could orient on one or both centers. In most instances the nuclei oriented on the nearest center only, and unipolar spindles formed when the nuclear envelope broke down. In a small number of experiments there was little separation of the centers, and bipolar spindles formed. In these experiments the farther center was either close enough to interact with the nucleus or with nuclear material following nuclear envelope breakdown.

Fig. 1 shows photographs of two sister cells after treatment with 1×10^{-6} M Colcemid and centrifugation. Centrifugation has moved the nuclei to the centripetal pole and caused some stratification of granular elements. In Fig. 1a asters are not readily apparent other than as granule-free areas. After irradiation at 366 nm in the region circled, two small, well separated birefringent centers appeared in that cell and became more obvious with time (1b). The nucleus in the irradiated cell then moved to the nearer pole (1b, 1c, 1d). Further irradiation, this time of both cells, led to the recovery of birefringent centers in the other cell (1d). In this cell the centers were initially close together but subsequently moved apart (1e, 1f) and the nucleus again moved to the nearer center. Nuclear membrane breakdown occurred at the same time in both cells and was followed by the formation of unipolar spindles involving only one center in each cell (1g).

These unipolar spindles maintained some of their birefringence much longer than would occur in bipolar spindles and at least until that time

[2] The separation of a nucleus from its centers is considered to result from a balance of conditions in which an aster which has been weakened and reduced in size by Colcemid still retains enough structure to remain anchored in the cytoplasm while the nucleus is moved away by centrifugation. If the nucleus has been moved far enough away and for a long enough time for the aster to be further reduced in size, the separation is stable until the aster is allowed to reform. Comparatively minor variations in eggs or in experimental procedures might be expected to affect the frequency of separation greatly.

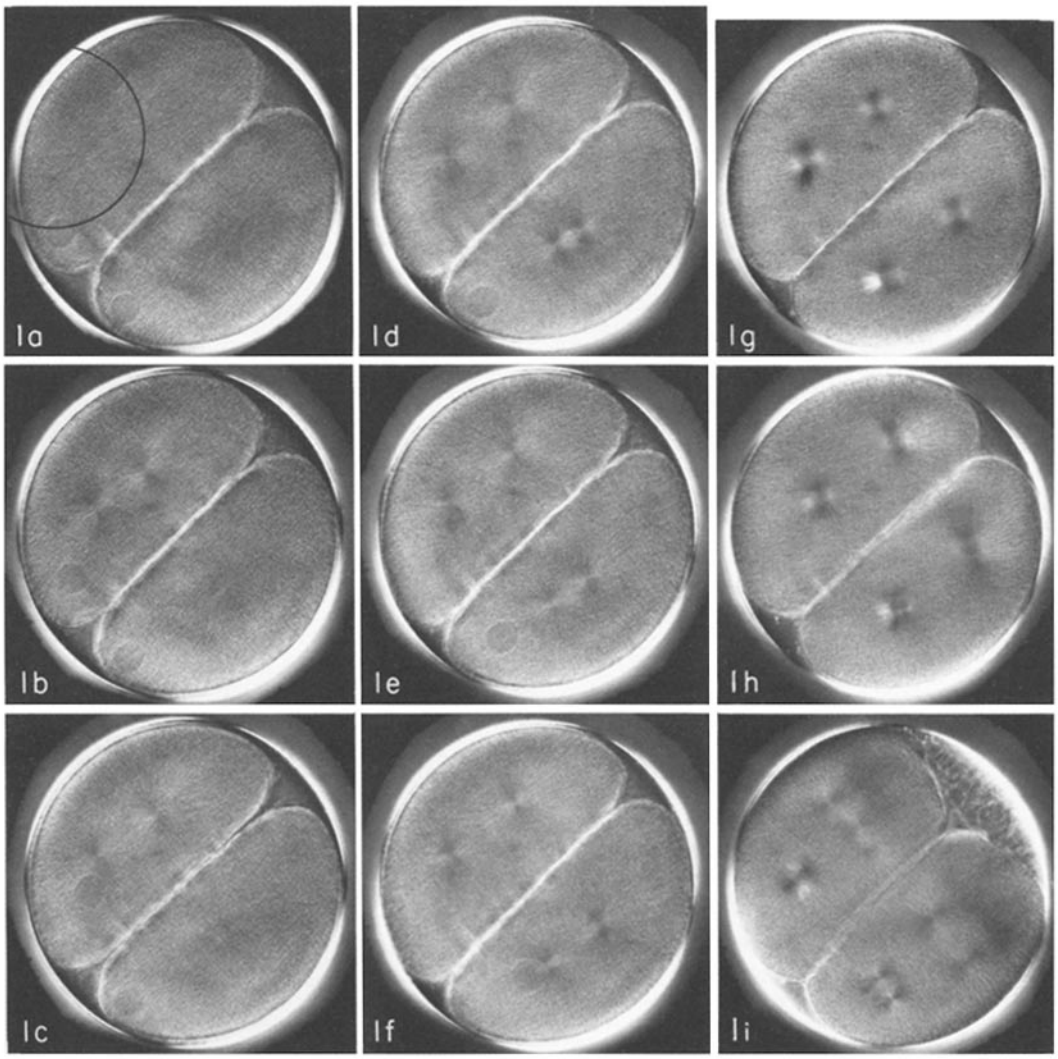

FIGURE 1 Eggs from a preparation with 20% of the eggs cleaved were placed in 1×10^{-6} M Colcemid 56 min after fertilization. Approximately 10 min later they were centrifuged at a maximum force of 30,000 *g* for 3.5 min and transferred to Colcemid-free sea water at 73 min after fertilization. Polarized light. (*a*) Preirradiation photograph showing the region (within the circle) to be irradiated at 366 nm. Irradiation for 1 min started 79.5 min after fertilization, and this was considered to be time (0). (*b*) 1.8 min. (*c*) 4.8 min, both cells were irradiated with 366 nm light for 1 min at 7.5 min. (*d*) 9.5 min. (*e*) 11 min. (*f*) 11.8 min. (*g*) 23 min, about 3 min after nuclear membrane breakdown. (*h*) 43 min, marked decrease in spindle fiber birefringence finally. (*i*) 50.5 min, the centers have separated except where spindle birefringence is still visible and the cortex has begun to break up. × 250.

when the centers separated again. Separation of centers (1h, 1i) occurred at the same time and somewhat more clearly in the "bare" centers than it did in the centers with nuclear material. After this, the cortex fragmented badly at the centrifugal pole in both cells.

Fig. 2 shows photographs from a similar experiment, again showing 366 nm dependent recovery of astral birefringence followed by movement of the nuclei to the nearer pole (2a, 2b, 2c, 2d, 2e, and 2f). However, in this experiment the nucleus in the first cell irradiated oriented on both centers, giving a normal bipolar spindle which led to cell cleavage. A unipolar spindle formed in the other

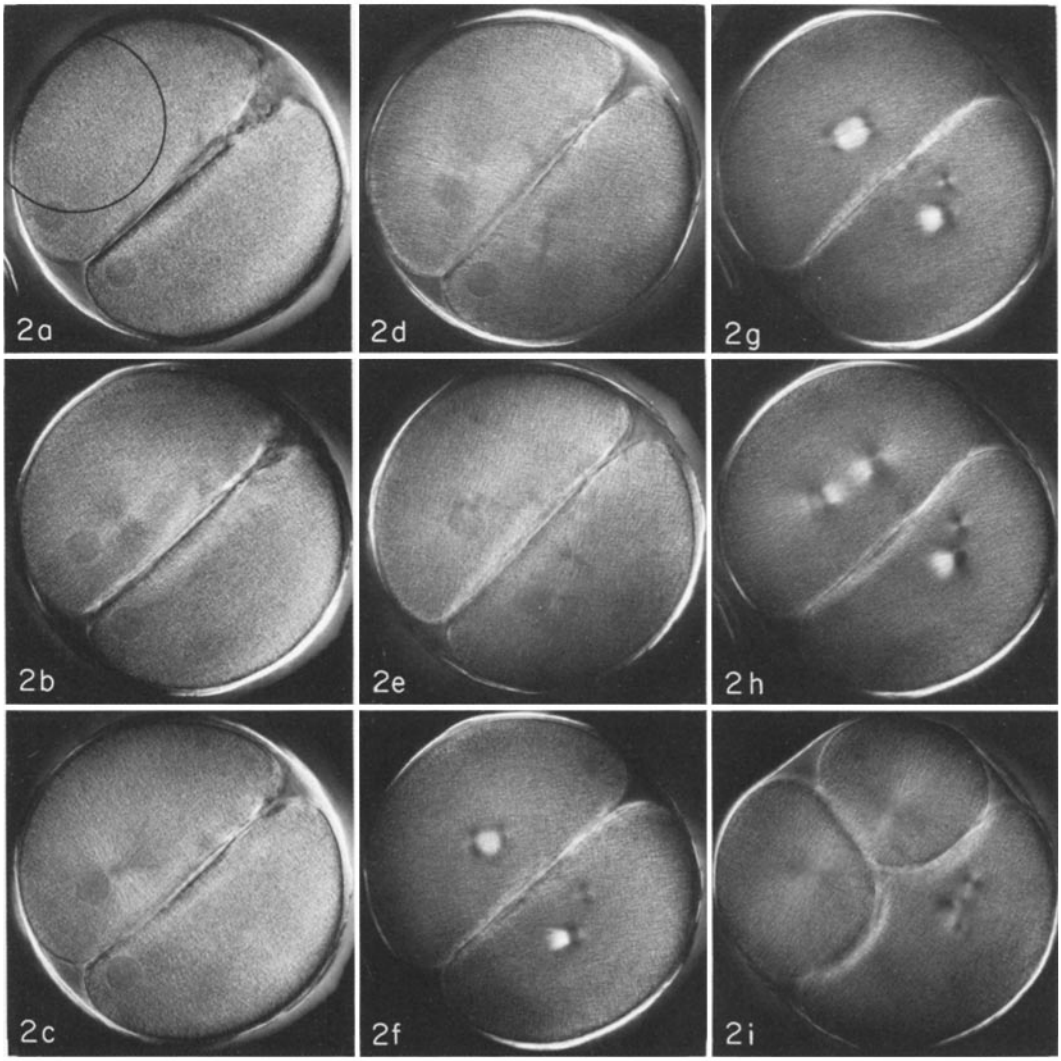

FIGURE 2 Eggs were placed in 1×10^{-6} M Colcemid 50 min after fertilization and then in calcium-free artificial sea water 15 min later after centrifugation for 3.5 min at a maximum force of 30,000 *g*. Polarized light. (*a*) Photograph showing the region (within the circle) to be irradiated at 366 nm. Irradiation for 2 min was started 77 min after fertilization, and this was considered as time (0). (*b*) 5.3 min. (*c*) 5.5 min, both cells were irradiated for 1 min with 366 nm light. (*d*) 9 min. (*e*) 10.3 min. (*f*) 17.5 min, which was about 2 min after nuclear membrane breakdown. (*g*) 23.5 min. (*h*) 27.5 min. (*i*) 36.5 min. × 250.

cell. Nuclear membrane breakdown and decrease in spindle birefringence followed a grossly similar course in both spindles, although a remnant of unipolar spindle birefringence was apparent until well after cleavage of the cell with the bipolar spindle.

366 nm dependent movement of a nucleus to a center was observed in at least 30 cells and was demonstrable in almost all cells in which separation of the nucleus and the centers was observed. The exceptions may have been related to change in the nuclear envelope near the time of prometaphase.

DISCUSSION

These observations show that there is a Colcemid-sensitive motile system acting between the nucleus and a center at the two-cell stage in *Lytechinus*

variegatus. It is presumed that this system is present in other animal cells, and possibly in plant cells, where it acts dynamically to maintain an association between nuclei and centers. A variety of cell inclusions are moved by Colcemid-sensitive motility (6, 9), and the demonstrated nuclear movement may be one more example. More specifically, the motile system described here may be identical with the motile system which moves the female pronucleus following fertilization (7), in which case its properties can be studied without centrifugation and with and without Colcemid.

Centrioles are found at varying distances from the nuclear envelope. This suggests that the separation between the center and the nucleus may be actively determined by a balance between a rigid or pushing element, possibly the electron microscopically visible microtubules (4, 5), and a pulling force of the sort demonstrated in this study which is exerted in parallel. Since the nuclear envelope is deformable, this balance of forces may be reflected in the shape of certain nonspherical nuclei such as those of lymphocytes, neutrophils, and spermatids.

The unsolicited appearance at prometaphase of unipolar spindles and free asters is interesting enough to be commented on but may be complicated by the experimental procedures used. The birefringent fibers in the unipolar spindles did not splay out greatly, suggesting lateral interaction, and they are about the length expected of a half spindle fiber but with some variation among fibers. A sharp metaphase plate was not seen. The length of the birefringent fibers in the unipolar spindle shown in Fig. 2 decreased, and most of the birefringence was lost only slightly later than in the sister cell with a bipolar spindle. However, in most cells with unipolar spindles the birefringence stayed up longer than it did in control cells with bipolar spindles.[3]

Akinetic chromosome fragments in grasshopper neuroblast cells move poleward at late anaphase and are often incorporated into the nucleus (10). Since the nuclear envelope has been observed to reform about grasshopper chromosomes during late anaphase (11), it is reasonable to consider that this movement is also an example of the interaction between a center and nuclear membrane-bounded chromatin. If such a motile interaction between the nuclear envelope and the center exists during late anaphase in all cells, it would provide a mechanism for collecting and keeping chromosomes together as the spindle apparatus is disassembled and the nucleus reformed. This karyomere-collecting role of the center could be especially important for those cells in which chromosomes seem capable of organizing their own spindles (12).

[3] S. Inoué, H. Sato, and D. Mazia have also observed examples of both kinds of behavior in preparations of mercaptoethanol-induced half spindles (personal communication).

This paper is dedicated to the memory of Jack Schultz (1904–1971), a man whose ideas, as a friend and teacher, are surely present in this work.

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