THE FINE STRUCTURE OF PRONUCLEAR FUSION IN THE COENOCYTIC MARINE ALGA *BRYOPSIS HYPNOIDES* LAMOUROUX

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During the late nineteenth and early twentieth centuries many studies were made of fertilization in algae (2–4, 8, 9, 13, 16). Relatively few investigations have been presented that deal with the details of such a phenomenon at the fine structural level. Among those researches which have concerned themselves with gamete union (2, 3, 8), only one (2) has presented any details of pronuclear fusion. It is the purpose of the present paper to relate some fine-structural details of pronuclear fusion in a coenocytic marine green alga, *Bryopsis hypnoides*.

MATERIALS AND METHODS

Bryopsis hypnoides Lamouroux was collected in the vicinity of Woods Hole, Mass., during the months of June, July, August, and September, and placed in culture dishes. Usually within 48 hr of collection, gametogenesis had commenced and the plants could be separated according to sex. Motile gametes, released into sea water in the culture dish, were passed through Whatman #1 filter paper to remove debris.

Gametes of both sexes were combined and aliquots were taken at various times up to 65 min after mixing. Two methods of taking samples were used: 1.) Aliquots were poured into 3% glutaraldehyde in sea water, centrifuged to a pellet, solidified in a drop of agar, and then passed through subsequent steps; 2.) Taking advantage of the phototactic response of fusing gametes, the author placed pieces of polymerized Epon in the culture dish near the illuminated side of the dish. The fusing gametes then attached to this surface. The blocks of Epon were then placed in

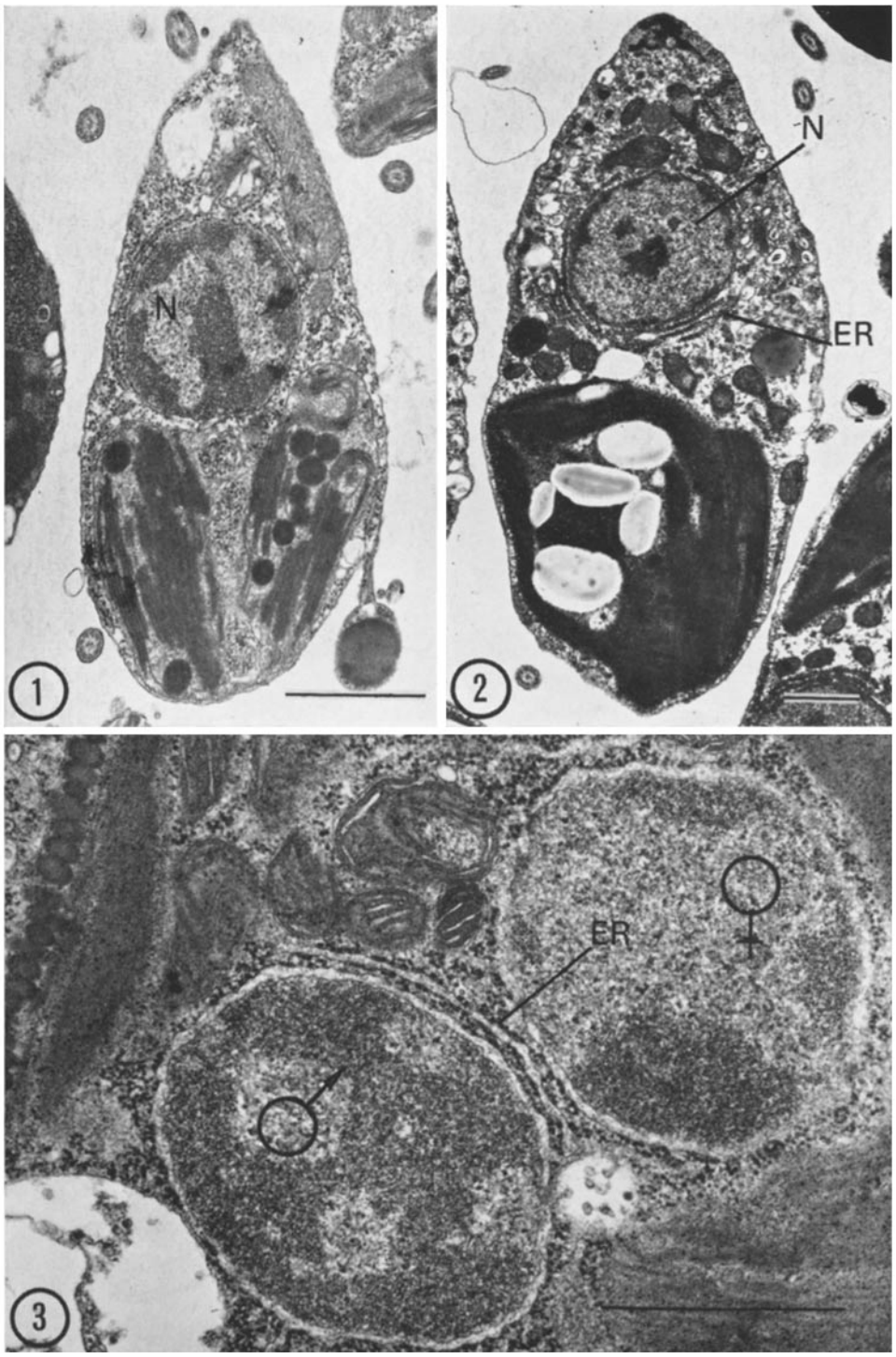

FIGURE 1 Electron micrograph of longitudinal section through male gamete. Note arrangement of chromatin in nucleus (*N*). × 19,600. Scale lines on all figures represent 1 μ.

FIGURE 2 Longitudinal section through female gamete. Note arrangement of chromatin in nucleus (*N*), and endoplasmic reticulum (*ER*) surrounding nucleus. × 10,700.

FIGURE 3 Section through zygote showing male (♂) and female (♀) pronuclei separated by a single cisterna of endoplasmic reticulum (*ER*). × 34,600.

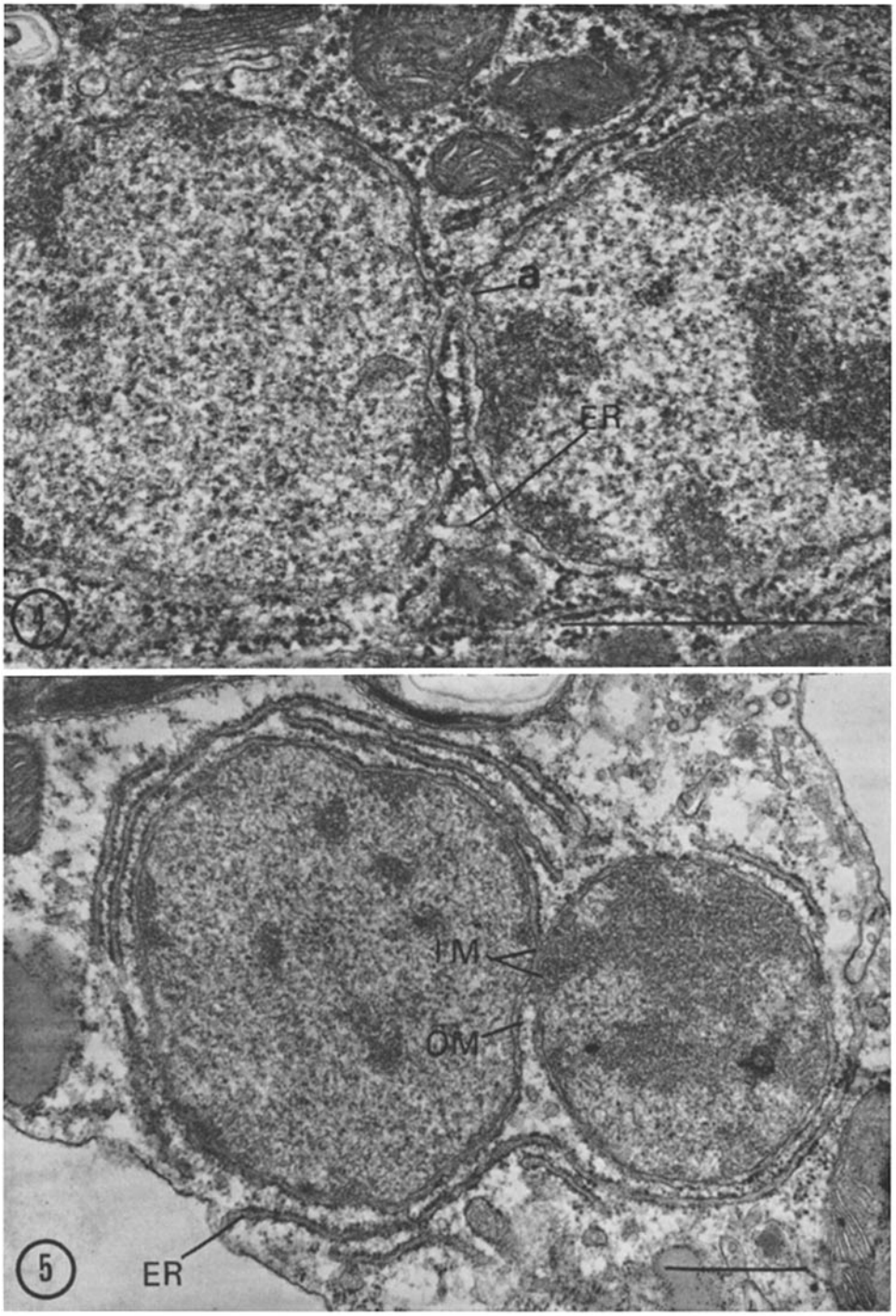

FIGURE 4 Section illustrating endoplasmic reticulum connection (*ER*) between two pronuclei. Note connection already established at (*a*). × 42,700.

FIGURE 5 Section through fusing pronuclei. Outer pronuclear membranes (*OM*) have fused, but inner membranes (*IM*) are still intact. Note endoplasmic reticulum (*ER*) surrounding both pronuclei. × 19,500.

glutaraldehyde and processed through subsequent steps. Male and female gametes were also collected by method 1 prior to mixing.

Following fixation for 2.5 hr in 3% glutaraldehyde in sea water, fusing gametes were postfixed for 1.5 hr in 1.33% OsO_4 in phosphate buffer, dehydrated in a graded series of ethanol, and infiltrated and embedded in Epon (7). Thin sections were stained initially with uranyl acetate followed by lead citrate (15), and examined with the AEI 6B, Hitachi HU-11E, and RCA EMU-3H electron microscopes.

OBSERVATIONS

Gametes

Gametes of *Bryopsis hypnoides* possess several features distinctive of their sex. Male gametes (Fig. 1) are 5 μ in length, 2.5 μ at their widest point, and have a nucleus 1.5 μ in diameter. A large portion of the nucleus is occupied by densely staining, granular chromatin. The outer lamina of the nuclear envelope is studded with numerous ribosomes. Rarely does more than one profile of endoplasmic reticulum surround the nucleus.

Female gametes (Fig. 2) are larger, 10 μ in length, and 4 μ at the widest point; the nucleus is 3 μ in diameter. Chromatin in the female nucleus appears as small aggregations. The density and degree of granularity is approximately the same as that observed in the male nucleus. The nuclear envelope is studded with ribosomes as in the male gamete, but the outer membrane of the nuclear envelope proliferates into larger amounts of rough endoplasmic reticulum, with typically three or four parallel stacks of cisternae. Nuclear pores are not present in male or female pronuclei, nor are intranuclear annulate lamellae as seen in *Arbacia* (6).

Pronuclear Fusion

After gamete fusion, each pronucleus is surrounded by rough endoplasmic reticulum. Even when pronuclei are separated by a distance of about 0.15 μ, a profile of endoplasmic reticulum intervenes between the pronuclei (Fig. 3). Unlike the situation occurring in *Arbacia*, there is no breakdown of the nuclear envelope of the male gamete. As the pronuclei approximate one another, a cisterna of endoplasmic reticulum connects one nuclear envelope with the other (Fig. 4). Thus, the perinuclear spaces of the two pronuclei become confluent. Continued approximation shortens the connecting cisterna of endoplasmic reticulum until the outer lamella of the one pronuclear envelope touches the outer lamella of the other pronuclear envelope. At this point these two outer lamellae fuse (Fig. 5), and this fusion is followed by the fusion of the two inner lamellae. A cisterna of endoplasmic reticulum is seen to surround the fusing pronuclei (Fig. 5). It is not known whether this cisterna is the result of movement of a single profile into a new position, or represents fusion of the endoplasmic reticulum components surrounding both pronuclei. Fusion of the inner lamellae of the pronuclear envelopes produces an internuclear bridge (Fig. 6). Successive widening of the internuclear bridge results in the zygote nucleus (Fig. 7). The chromatin of the zygote nucleus, whose envelope is composed of the original envelopes of the male and female pronuclei, becomes scattered, and it is not possible to distinguish the chromatin contributions from each gamete.

DISCUSSION

While the phenomenon of membrane fusion may be thought of as a general one, the data obtained in the present study coupled with the published information on pronuclear fusion in other organisms permits one to classify the fusion of membranes into two types. I shall refer to these types as direct and indirect. The present study of pronuclear membrane fusion in *Bryopsis* offers an example of indirect fusion, referred to as such since the fusion is effected by an intervening profile of rough endoplasmic reticulum. An indirect type of pronuclear membrane fusion appears to be the case in higher plants such as cotton, *Gossypium hirsutum* (5).

Although the vegetative morphology of *Bryopsis hypnoides* (14) is very different from that of *Chlamydomonas* (11, 12), gametes of both organisms are quite similar: they are biflagellate, uninucleate, chlorophyllous, and lack a cell wall. Pronuclear membrane fusion in *Chlamydomonas* is direct and like that which occurs in the sea urchin *Arbacia punctulata* (6), i.e., fusion is not initiated by a cisterna of the endoplasmic reticulum.

The significance of the direct and the indirect membrane fusion is unknown. One could, however, view the fusion of the pronuclei that occurs with the assistance of a cisterna of the endoplasmic reticulum as an efficient mechanism to minimize the radius of curvature of the pronuclei. Such a hypothesis finds some support in Pethica's theory of cell adhesion. Pethica (10) states that "barriers to adhesion between approaching cells indicate that villi and other small membrane protuberances are the most likely regions of initial contact." In

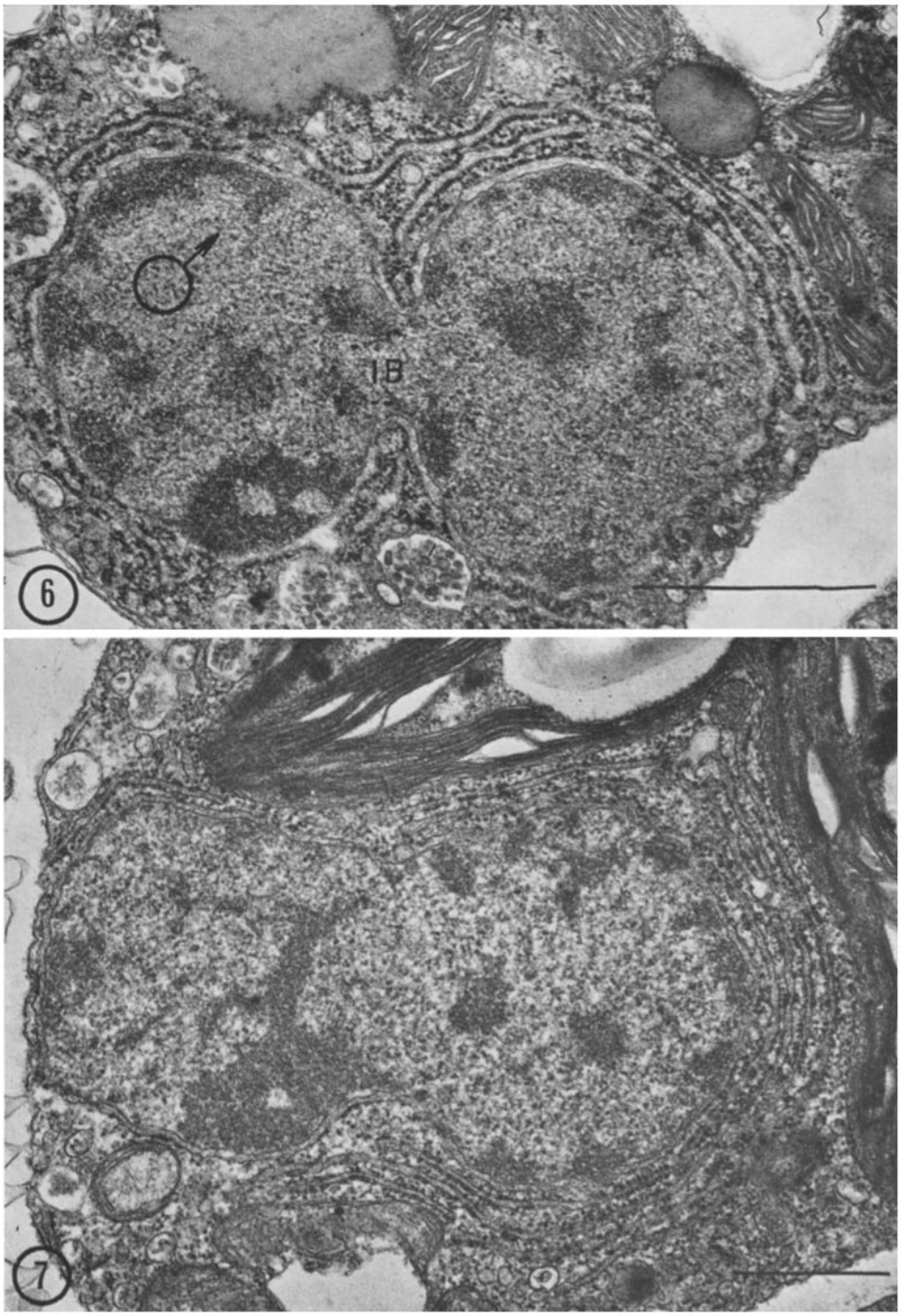

FIGURE 6 Section through internuclear bridge (*IB*). Characteristic chromatin of male pronucleus (♂) is still discernible. × 33,600.

FIGURE 7 Section through zygote nucleus. × 21,500.

Bryopsis the endoplasmic reticulum profiles have a tip diameter of 0.05 μ and thus would be a very favorable instrument for initial contact, the requirement being a maximum of 0.2 μ diameter for membranes to approach within 5–10 A (a distance suitable for ionic bridging) (1).

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