OBSERVATIONS ON THE FUSION OF NUCLEI IN PLANTS

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During the life cycle of the flowering plant, there are normally three occasions when nuclei fuse: (1) when the two polar nuclei of the mature megagametophyte fuse; (2) when one of the two male nuclei fuses with the egg nucleus; (3) when the second male nucleus fuses with the combined polar nuclei. Observations on the actual fusion of the nuclei made with the light microscope are vague and conflicting (Maheshwari, 1950; Sharp, 1943; Vazart, 1958). Recent observations made with the electron microscope and reported here have revealed the basic method of nuclear fusion in the cases mentioned above.

MATERIALS AND METHODS

The plant used was cotton (*Gossypium hirsutum*). The nucellus was isolated at various times after the flower had opened and had been hand pollinated. The tissue was fixed in 2 per cent $KMnO_4$ at 4°C for 4 hours or in 6 per cent glutaraldehyde (pH 6.8, 0.1 M phosphate buffer) at 4°C for 4 hours, washed in buffer, and placed in 2 per cent OsO_4 at 4°C for 12 hours. The dehydration was through an acetone series. To the 70 per cent acetone was added 1 per cent uranyl nitrate. All tissue was embedded in Epon and sectioned with a diamond knife. All observations were made with a Bendix Transcope RS-50-E-1.

OBSERVATIONS

The nuclei were observed to fuse in the following manner. The nuclei which will fuse migrate toward one another, but a layer of cytoplasm, including mitochondria, Golgi apparatus, spherosomes, endoplasmic reticulum, and even small vacuoles, still separates them (Figs. 1 (*A*) 2). Next, at several points elements of the endoplasmic reticulum from the two nuclei fuse, making the outer nuclear membranes of the two nuclei continuous (Figs. 1 (*B*), 2 (arrows)). The membranes connecting the two nuclei apparently decrease in length and membranous bridges composed of the outer nuclear membranes are formed (Figs. 1 (*C*), 3). The inner nuclear membranes next come into contact and merge (Figs. 1 (*D*), 3). Thus, first the outer and then the inner nuclear membranes fuse

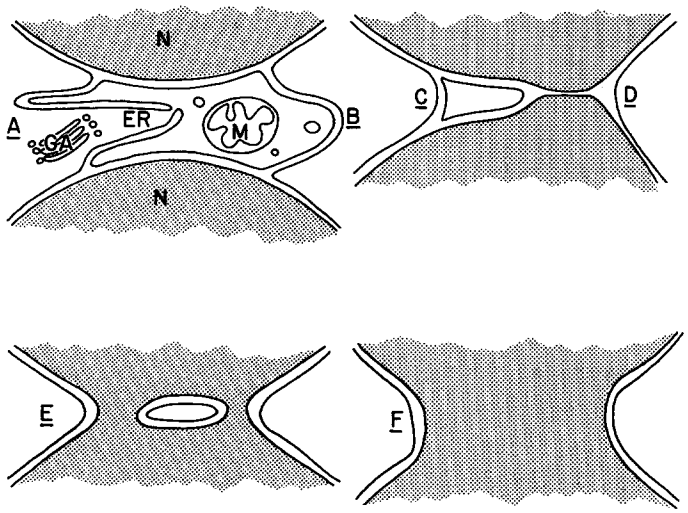


FIGURE 1 Diagrammatical representation of the mechanism of nuclear fusion in plant nuclei. *A*. Conditions before the beginning of nuclear fusion; *B*. Fusion of the elements of the endoplasmic reticulum (*ER*) connected to the nuclei; *C*. Shortening of the connected *ER*; *D*. Coming together of the inner membranes; *E*. Formation of many small bridges; *F*. Joining of the small bridges to form a large, final central bridge between the two nuclei. *N* = nucleus; *M* = mitochondrion; *ER* = endoplasmic reticulum; *GA* = Golgi apparatus.

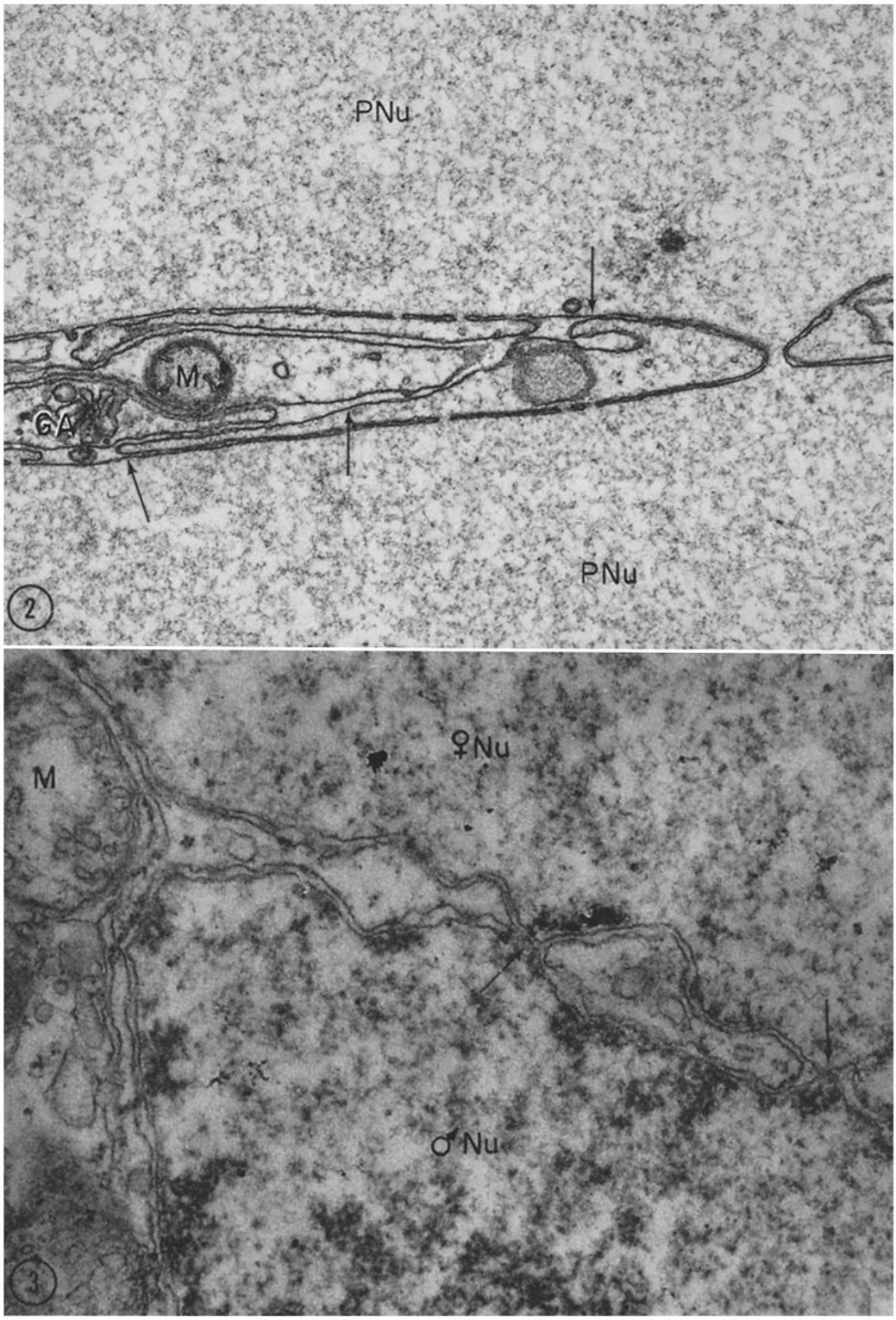
and a complete bridge between the two nuclei is formed (Figs. 1 (*E*), 2). The bridges are numerous and give the impression, in sectioned material, that cytoplasmic organelles are trapped between the nuclei. This, however, does not appear to be true, as the cytoplasm between the nuclei is continuous throughout the region. The nuclear bridges enlarge and appear to coalesce (Fig. 1 (*F*)), pushing the cytoplasmic elements out as they do so. When the nuclei are completely fused there is no evidence of the presence of entrapped cytoplasm.

This description of nuclear fusion in plants is based upon six cases of fusion of polar nuclei, one of fusion of an egg and sperm nucleus, and one of fusion of a sperm nucleus and the fused polar nuclei. As it requires approximately 36 hours for the polar nuclei to fuse, a more complete sequence of events can be developed in their case. For example, one preparation showed the nuclei connected only by the endoplasmic reticulum, while another, 8 hours later, showed bridges as well as connected endoplasmic reticulum, and a still later preparation had the nuclei joined by a large central bridge and several small peripheral ones. Thus, through a time study of this type it was possible to reconstruct the sequence of events in the fusion of the polar nuclei. The fusion of the male nuclei with the egg and the already fused polar nuclei follow the same pattern.

The observations give no evidence that any mass breakdown of the nuclear membrane occurs. The data, however, suggest that the membranes do contract possibly through some localized area of breakdown. No evidence was found indicating the

FIGURE 2 Fusion of two polar nuclei. The arrows indicate examples of the endoplasmic reticulum bridging the two nuclei. Also visible is a later stage of fusion in which the inner and outer nuclear membranes are continuous. Tissue fixed in $KMnO_4$. *PNu* = polar nucleus; *M* = mitochondria; *GA* = Golgi apparatus. × 36,000.

FIGURE 3 Fusion of the egg nucleus and male nucleus. The outer nuclear membranes are joined and the inner nuclear membranes are close together (arrows). Tissue fixed in glutaraldehyde-OsO_4. ♀ *Nu* = egg nucleus; ♂ *Nu* = male nucleus; *M* = mitochondria. × 40,000.


PNu
M
GA
PNu
2
M
♀Nu
♂Nu
3

mechanism by which the membranes themselves fuse. In the case of the inner nuclear membranes, the two membranes are seen to be pressed closely together and then completely joined with no details of the intervening phase.

The present data indicate the importance of the nuclear membrane in the act of nuclear fusion in plants. The membranes of both nuclei contribute to the new nuclear membrane. This fact may prove to have genetic and developmental implications in the case of the fusion of the egg and the sperm nuclei in plants.

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