COTTON EMBRYOGENESIS

Polysome Formation in the Zygote

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The formation of the zygote in cotton is accompanied by marked changes in the number and aggregation of the ribosomes. The zygote is formed approximately 14 hr after pollination but does not divide for some 56–60 hr. Electron microscope studies of the zygote during this period reveal a series of changes in the size and distribution of polysomes which may provide information on this important stage of plant development.

MATERIALS AND METHODS

Ovules of *Gossypium hirsutum* L., variety M 8949, were collected at various times before and after pollination. They were fixed for 12 hr in cold 3% glutaraldehyde in 0.05 M cacodylate buffer, pH 6.8. The tissues were rinsed and postfixed in 2% OsO_4 for an additional 12 hr. After dehydration in acetone, they were embedded in Epon. The 70% acetone step of dehydration contained 1% uranyl nitrate. The sections were stained with lead citrate and examined with a Zeiss EM-9.

Nucelli were isolated from some of the ovules, and the ends were removed. These ends were then fixed in glutaraldehyde (6% in 0.06 M phosphate buffer) for 3 hr. After having been washed for 1 hr in water, they were placed in 0.1% RNase (Worthington Corporation, Harrison, N.J.) at pH 6.8 for 16 hr at 30°C. They were then washed in water, fixed for 4 hr in 2% OsO_4, dehydrated in acetone, and embedded in Epon. This procedure was a control on identifying the nature of the particles as ribosomes and confirmed the fact that in all cases the particles are ribosomes (Fig. 4).

RESULTS

The ribosomes of the egg (Fig. 1) are uniformly distributed throughout the ground cytoplasm or attached to the endoplasmic reticulum (ER). They are not found concentrated near plastids or mitochondria (Fig. 1). The degree of ribosomal aggregation appears low, and most ribosomes appear alone or in small polysomes.

A striking change in ribosomal aggregation and distribution occurs (Fig. 2) at the time of fusion of the egg and sperm nuclei. The ribosomes become grouped around the plastids and mitochondria. There they aggregate into long, helical polysomes. The length of these polysomes varies but as many as 25–30 ribosomes can be counted in some of the polysomes (Fig. 3). Few single ribosomes can be seen in the ground cytoplasm at the peak of this aggregation phase (Fig. 2).

The formation of these helical polysomes is correlated with the presence of the sperm nucleus and the beginning of nuclear fusion. The first helical polysomes are found near the fusing nuclei and later in the parts of the cell further from the nuclei. By the time that nuclear fusion is essentially complete, some 6–8 hr, all of the ribosomes appear to be aggregated into the helical polysomes. During the polysome formation stage there is no morphological evidence that additional ribosomes are being synthesized by the cell.

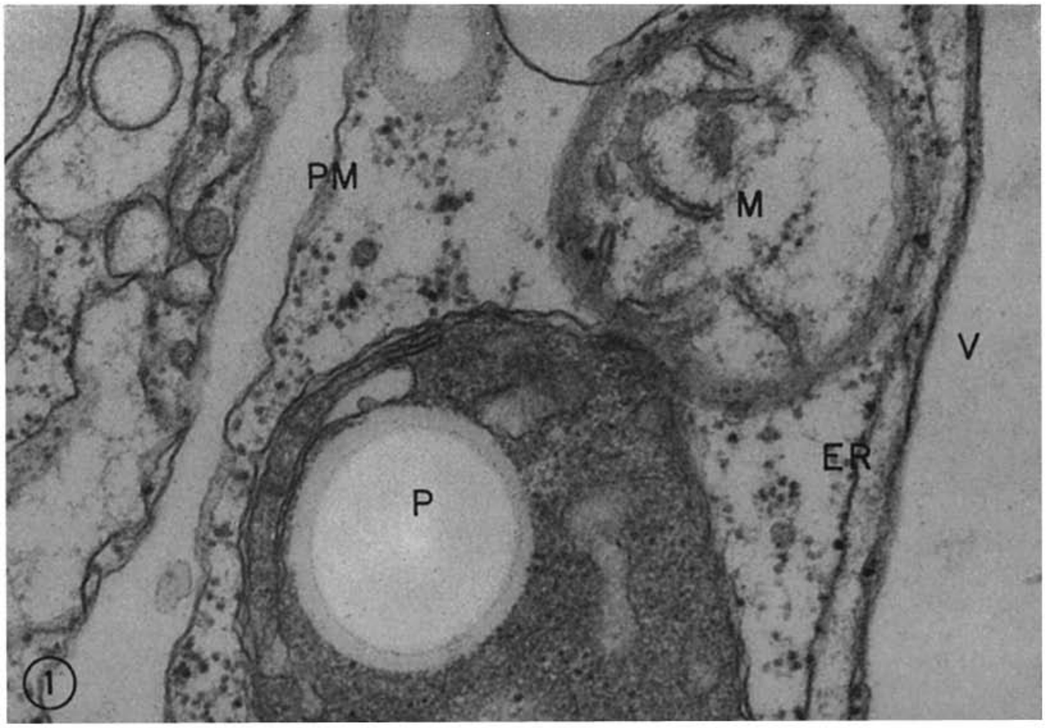
Approximately 12 hr after the zygote nucleus is complete (or roughly 30–35 hr after pollination) the number of ribosomes begins to increase. These ribosomes appear in the ground cytoplasm and either are alone or are aggregated into polysomes consisting of three to four ribosomes. There is no apparent change in the number of helical polysomes, and all types are present in the zygote at the same time (Fig. 3).

By the time that the zygote divides (some 72 hr after pollination), the small polysomes and single ribosomes are numerous in the cell (Fig. 5). Accompanying this increase in small polysomes and ribosomes is an increase in density of the ground cytoplasm (compare Figs. 2 and 5).

The helical polysomes persist through the first several divisions of the embryo but then disappear. They can no longer be seen by the globular stage although numerous small polysomes are present.

DISCUSSION

The data suggest the following interpretation: the helical polysomes formed during gametic fusion are composed of the ribosomes present in the egg; the stimulus for their formation is synthesized or released by the fusing nuclei and is presumably messenger RNA; the nature of this RNA together with other conditions or factors present in the zygote result in the helical form of the polysome;



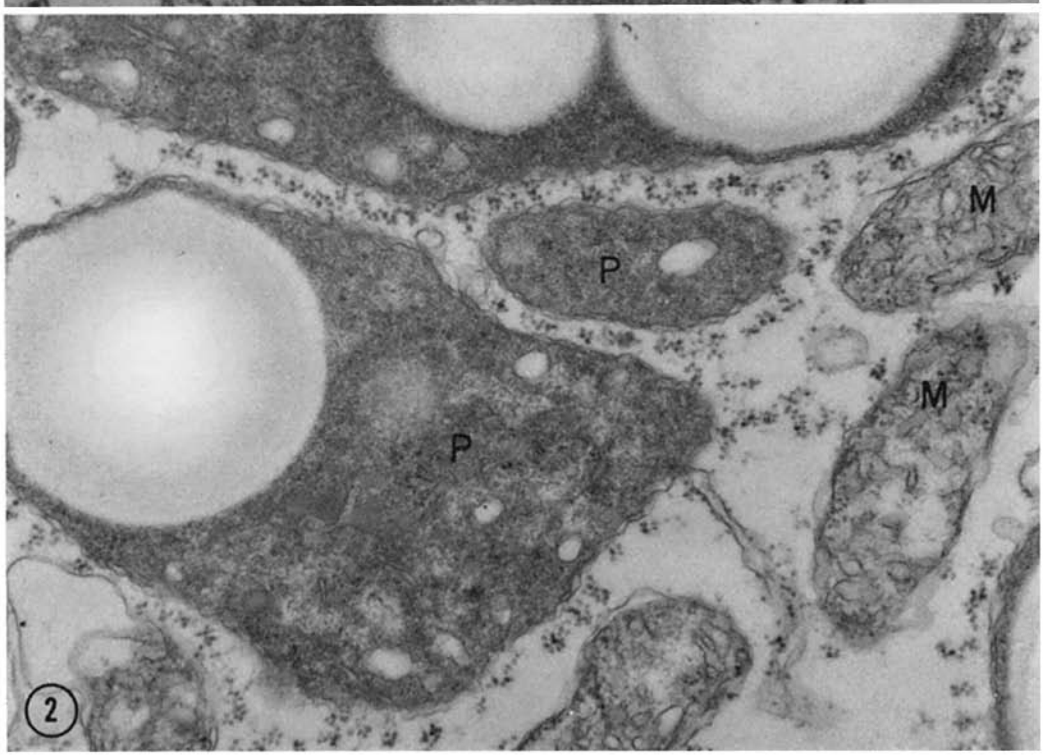


FIGURE 1 Ribosomes of the egg of cotton. The egg cytoplasm occupies the right two-thirds of the photograph; the central cell occupies the left one-third. *M*, mitochondria; *P*, plastid; *PM*, plasma membrane; *V*, vacuole; *ER*, endoplasmic reticulum. × 62,000.

FIGURE 2 Ribosomes of the zygote during nuclear fusion, 18 hr after pollination. Note distribution of polysomes around plastids (*P*) and mitochondria (*M*). × 42,000.

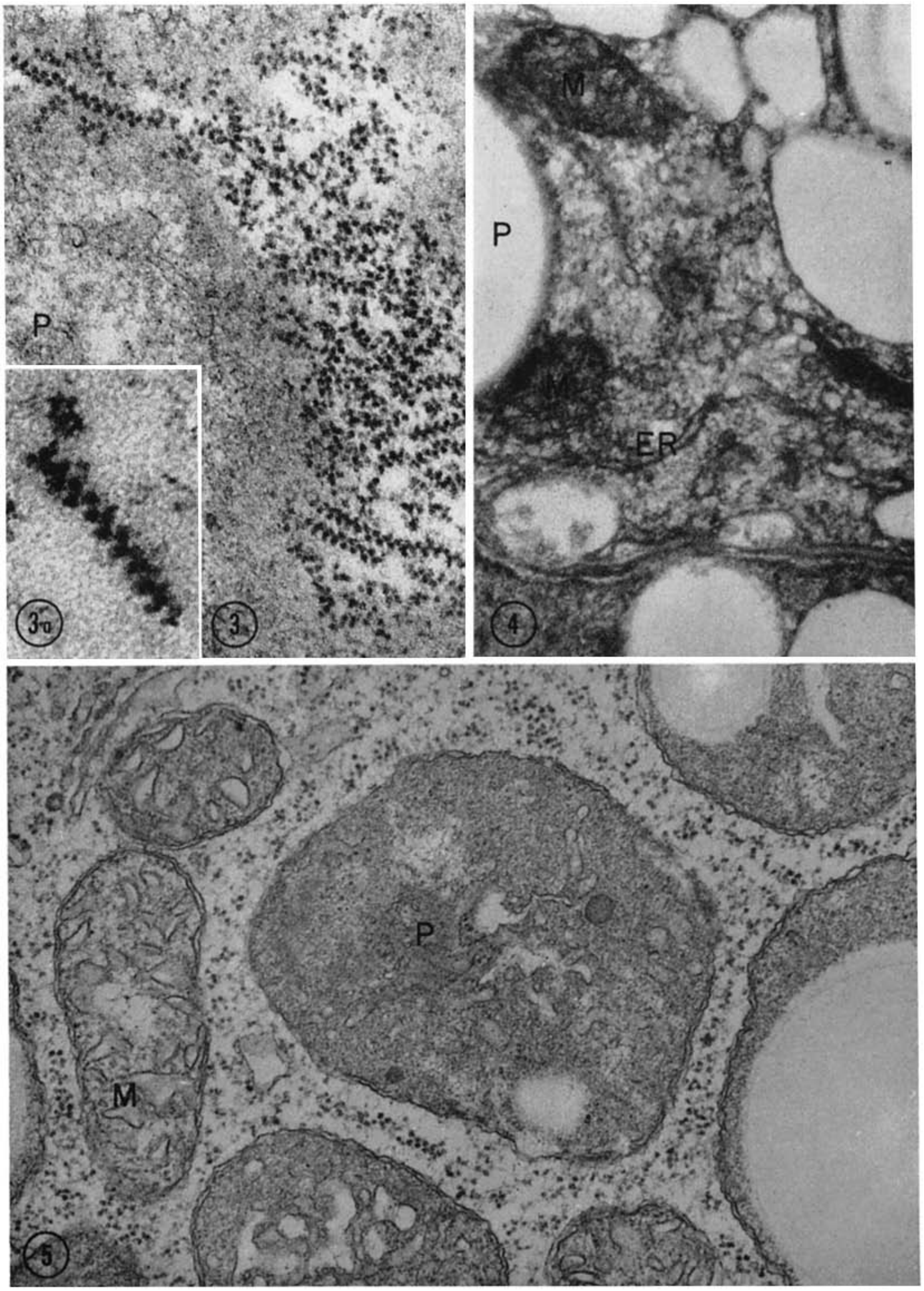


FIGURE 3 Helical polysomes associated with the surface of a plastid. The section is tangential to the surface of a plastid (*P*). 3 *a* Enlarged view of a helical polysome. Fig. 3, × 59,000; Fig. 3 *a*, × 118,000.

FIGURE 4 Portion of a zygote 24 hr after pollination which had been treated with RNase. Note the absence of ribosomes in the section. *M*, mitochondria; *ER*, endoplasmic reticulum. × 42,000.

FIGURE 5 Zygote during division. Both helical polysomes and small polysomes can be seen. × 42,250.

after nuclear fusion has been complete and the zygote nucleus has been organized, a new generation of ribosomes is produced; these new ribosomes form the short polysomes which are seen in the older zygote and which again presumably are formed on the basis of a messenger RNA now synthesized by the zygote nucleus; the nature of this RNA as well as the conditions present in the older zygote determine the shorter form of polysome. If this interpretation is correct, the zygote, by the time it divides, contains a mixture of two generations of ribosomes and at least two generations of messenger RNA.

The possibility exists that the long, helical ribosomes represent a type of informosome (4). Helical polysomes have been found previously in a number of animal and plant tissues (1–3, 5). They are always associated with some change in cell development, although those previously described have not been so long as those in the cotton zygote. Clearly the significance of the different kinds of polysomes cannot be definitely assigned until evidence on their role in protein synthesis has been collected. Fortunately, since the system is open to experimental work, such data can be obtained.

SUMMARY

The ribosomes of the egg of cotton show a low degree of aggregation into polysomes. In correlation with the beginning of fusion of the egg and sperm nuclei, these ribosomes become arranged into long helical polysomes. These helical polysomes are found surrounding the plastids and mitochondria. After the zygote nucleus has been formed, additional ribosomes appear in the cell. These ribosomes aggregate into small polysomes, but no additional helical polysomes appear to be formed. Both types of polysomes exist through the first divisions of the embryo, but then the helical polysomes gradually disappear. The possible relation of these changes in polysome population to plant embryo development is discussed.

This work was supported by grants from the National Science Foundation (GB 3460), the National Institutes of Health (5-R01-CA 03656-9), and the Miller Institute for Basic Science, University of California, Berkeley.

The assistance of Miss Mary Ashton, Mrs. Barbara Raymond, and Mrs. Paula Stetler is gratefully acknowledged.

Received for publication 18 August 1967, and in revised form 23 October 1967.

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