AN APPARENT HELICAL ARRANGEMENT OF RIBOSOMES IN DEVELOPING POLLEN MOTHER CELLS OF *IPOMOEA PURPUREA* (L.) ROTH

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Behnke (1963) has recently described a helical arrangement of ribosomes in differentiating cells of the small intestine of the rat fetus, and Waddington and Perry (1963) have found the same structures in the muscle cells of *Rana pipiens* embryo. In the course of an investigation into the development of pollen in *Ipomoea purpurea* (L.) Roth, a similar arrangement of ribosomes has been seen in the differentiating pollen mother cells. It is the purpose of this study to describe these structures which have not previously been found in plant tissue.

MATERIALS AND METHODS

Freshly excised buds of *Ipomoea purpurea* (the common morning glory), from which the bracts and scales had been removed, were placed in 1.5 per cent glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 16 hours at 4°C. The buds were washed several times in the same buffer and placed either in 2 per cent osmium tetroxide in Veronal-acetate buffer, pH 6.1 (Kellenberger *et al.*, 1958), or in 2 per cent osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, for 2 hours at 4°C. The buds were dehydrated through a graded acetone series, embedded in Araldite (Glauert, 1962), and sectioned with a diamond knife on a Huxley microtome. Light to dark grey sections, as viewed in reflected light, were picked up on uncoated 400-mesh copper grids. Sections were stained for 60 minutes in 1 per cent aqueous uranyl acetate, pH 5.0, washed well with distilled water, and placed in lead citrate stain (Reynolds, 1963) for 30 minutes. The grids were again thoroughly washed in distilled water, carefully dried and lightly coated with evaporated carbon, and examined in an A.E.I. EM 6 electron microscope.

In the initial stages of the work on the ontogeny of *Ipomoea* pollen, the tissue was not prefixed in glutaraldehyde and the helix-like arrangements of ribosomes were not seen in sections cut from this material, possibly due to the poor quality of fixation and embedding. Prefixation in glutaraldehyde was routinely used after it was found to increase the resolution and clarity of the micrographs, and reveal structures, such as the helix-like arrangement of ribosomes, which had not previously been seen in *Ipomea*. It is not presently clear whether these structures are an artefact due to

the presence of the glutaraldehyde or an enchancement of an as yet undiscovered structural detail.

OBSERVATIONS

The arrangement of the ribosomes is shown in Figs. 1 to 4. The double rows of ribosomes which are slightly staggered are approximately 350 A wide and formed from a series of ribosomes 165 to 175 A in diameter arranged at an angle of about 25° to the long axis. Because of the spacing of the individual particles and the estimated section thickness (200 to 300 A), it is assumed that these structures are a section through a helix and not just parallel rows of ribosomes. Ribosomes may appear in parallel rows when associated with the endoplasmic reticulum, but in this instance are not so tightly packed or staggered as in the helix-like structures. There is little doubt that the small electron-opaque objects are ribosomes, because they reveal the characteristic electron image after staining with uranyl acetate and lead citrate. Paraffin-embedded sections of *Ipomoea* buds stained with methyl green and pyronin (Kurnick, 1955) have confirmed the presence of large amounts of ribonucleic acid in both the developing pollen mother cell and surrounding tapetum.

The helix-like structures are confined to the cytoplasm of the developing pollen mother cell and are absent from the tapetal cells. This arrangement of ribosomes is the exception rather than the rule, and it is assumed that the structures are oriented at random in the cytoplasm or bent into other configurations and only appear when sectioned in a favourable plane.

The ribosomes of the pollen mother cell are generally not associated with the endoplasmic reticulum, but appear free in the cell cytoplasm as single or double rows, rosettes and aggregates of various sizes (Fig. 2.). This is in contrast with their appearance in the tapetal cells where they are invariably associated with the endoplasmic reticulum.

DISCUSSION

It is not presently clear whether the structures are analogous to the polyribosomes which have been found in a wide variety of tissues (Rich *et al.*, 1963; Warner *et al.*, 1962; Warner *et al.*, 1963; Bayley, 1964; Phillips *et al.*, 1964; Fitz-James, 1964). There is good evidence from these and other studies that in cells which are synthesizing protein the majority of the ribosomes are aggregated into polyribosomes of various sizes held together by thin strands of messenger ribonucleic acid. The number of ribosomes per polysome appears to bear some relation to the molecular weight of the protein being synthesized, and the distribution of polysome sizes within the cell is thought to be a reflection of the number of different proteins being manufactured.

Differentiating pollen mother cells would appear to be a tissue in which polysomes are likely to be found. The cells are undergoing rapid division and differentiation to form the microspores, which would require the synthesis of large amounts of different proteins.

Assuming that the helix-like structures appear only when sectioned in a favourable plane, they are still comparatively rare within the cell. If these structures are morphologically equivalent to polysomes, then they are probably engaged in biosynthesis to produce a protein at relatively low concentration in the cell.

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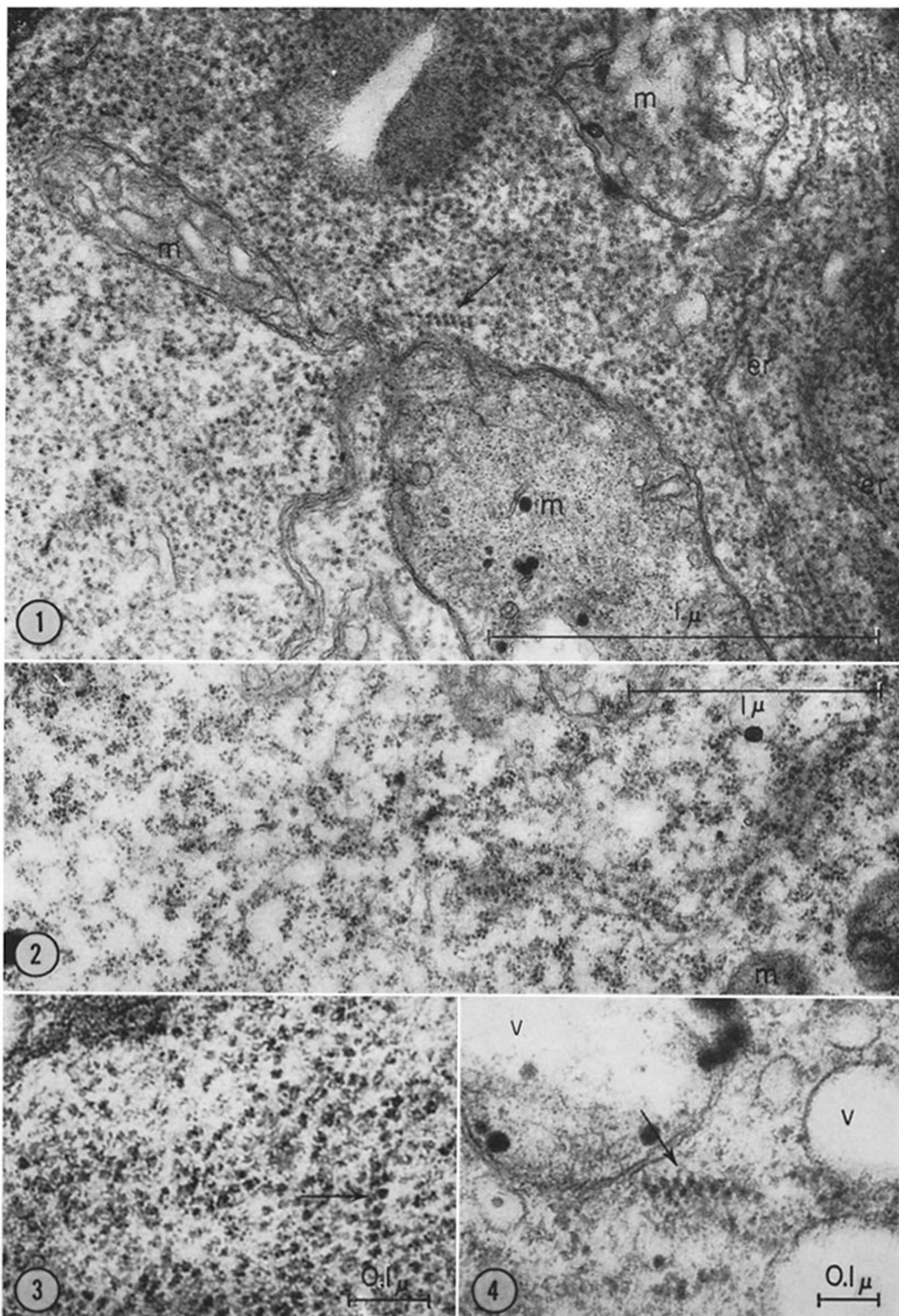
FIGURES 1 to 4 Micrographs showing sections through the cytoplasm of differentiating pollen mother cells of *Ipomoea purpurea* (L.) Roth. The arrows indicate ribosomes arranged in apparent helices. The scale in Figs. 1 and 2 indicates 1 μ. *er*, endoplasmic reticulum; *m*, mitochondrion; *v*, vacuole.

Fig. 1: Fixation in glutaraldehyde followed by phosphate-buffered osmium tetroxide. × 60,000.

Fig. 2: Fixation in glutaraldehyde followed by Veronal-acetate–buffered osmium tetroxide. × 39,000.

Fig. 3: Fixation in glutaraldehyde followed by phosphate-buffered osmium tetroxide. × 115,000.

Fig. 4: Fixation in glutaraldehyde followed by phosphate-buffered osmium tetroxide. × 92,000.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