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Cytoplasmic differentiation during microsporogenesis in higher plants

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Abstract

Conspicuous cytoplasmic dedifferentiation in the pollen mother cells takes place early in the meiotic prophase of many plants. This event involves the removal of much of the cytoplasmic RNA, and the differentiation of both plastids and mitochondria to approaching the sole expression of their genomes. Much of the RNA removed from the cytoplasm passes to the nucleoplasm where it is utilised in the construction of a new 'generation' of ribosomes. These new ribosomes are incorporated into cytoplasmic 'nucleoloids', which disintegrate in the post-meiotic cytoplasm, restoring its ribosomes to pre-prophase levels. These changes are interpreted as evidence of a process by which the cytoplasm is cleansed of sporophytic control elements, both for the expression of the new gametophytic genome, and in the female cells of higher plants, for transmission to the new generation. The absence of control elements (presumably long-term messenger RNA) from the cytoplasm would result in the dedifferentiation observed in the organelles, and the low levels of reserves in these cells presumably results in characteristically lengthy and unusual redifferentiation of both plastids and mitochondria, once information-carrying molecules again enter the cytosol.

INTRODUCTION

In view of the significance of events taking place in the nucleus during meiosis, it is hardly surprising that by comparison little attention has been focussed upon the changes that take place in the cytoplasm at this time. However, as early as 1920 Guillermond noted that cyclic changes were occurring in the "chondriosome" of pollen mother cells of *Lilium*. The resolution of techniques available at that time made interpretation of these cycles almost impossible, such that Wagner (1927) saw them as indicating discontinuity in the lines of elements of the chondriosome — now known to be organelles, whereas Guillermond (1924) suggested that these changes demonstrated the continuity of line of these elements. Further work by Py (1932), a student of Guillermond, showed that some further, so-far undetected, change over-

came the cytoplasm such that its 'stainability' also changed during meiosis. Painter investigated these differences in staining quality in 1943, and working with *Rhoeo discolor*, was able to show that they resulted from a decrease in basophilia of the cytoplasm.

That the cytoplasmic changes during meiosis were highly specialised was underlined by work by Gaudavan and Chin Chen (1936) who demonstrated the presence of nucleolar-like bodies in the cytoplasm of cells closely following telophase II, about the time the cytoplasm regained its full basophilia. The use of the electron microscope did little to simplify matters; Bal and De (1961) investigated the meiotic cytoplasm of *Tradescantia* and reported that while the presence of mitochondria was continuous throughout development, plastid line was discontinuous, these organelles being regenerated from 'large pleomorphic bodies' in the cytoplasm of the tetrad. These surprising events appeared also to be paralleled in the lower plants, for Bell and Mühlethaler (1964) described the generation of a new population of organelle-like inclusions from the nuclei of developing egg-cells of *Pteridium*.

More recently, investigations with improved techniques have revealed that while in higher plants continuity of organelle line is maintained (Marumaya, 1968; Dickinson, Heslop Harrison, 1970a), and much of the 'chondriosome cycles' may be explained in terms of the distribution of lipid droplets (Dickinson, Heslop Harrison, 1968), substantial changes certainly to take place in the meiocyte cytoplasm, many related to a cycle of RNA metabolism (Mackenzie et al., 1967). Details of this cycle, and of other events accompanying meiosis are presented here, and their significance discussed in the light of the eventual fate of the gametophytic cytoplasm.

THE EVENTS PRIOR TO MEIOTIC PROPHASE

That a dedifferentiation of the premeiotic cytoplasm takes place was confirmed by Mackenzie et al. (1967) in work which showed a rapid drop in visible ribosome numbers and extractable cytoplasmic RNA during early prophase. This also served to explain the results of Py (1932) and Painter (1943), in that the 'decrease in basophilia' described could be correlated with the removal of at least, ribosomal, RNA from the cytoplasm. Precise details of the fate of this RNA, once degraded, are not known, but preliminary tests suggest that it moves to the nucleus, where it forms part of a new generation of ribosomes, or other species of RNA (Mackenzie, 1967; Williams, 1971).

A drop in visible ribosomes, or microphotometrically detected RNA has now been reported as occurring in many plants (Figs 1, 2), ranging from unicellular algae (Siersma, Chiang, 1971) and ferns (Shef-

field, 1978), to both monocotyledons (e.g. *Lilium*, Mackenzie et al., 1967) and dicotyledons (e.g. *Cosmos*, Knox et al., 1970). The extent to which cytoplasmic RNA is degraded varies considerably between plants. In *Lilium* very few ribosomes are visible in the early leptotene cytoplasm, while in *Pinus*, the difference in ribosome frequency is hardly detectable (Dickinson, Bell, 1976). Attempts to identify the agent responsible for the eradication of cytoplasmic RNA have largely been frustrated. Certainly, the disappearance of ribosomes and cytophotometrically-detectable RNA can be correlated with the appearance of non-specific acid phosphatase. But further attempts to characterise this enzyme, and indeed, to examine levels of RNAase in these cells have produced very equivocal results.

The dedifferentiation of the premeiotic cytoplasm is, however, not restricted to RNA. As suggested by the work of Bal and De (1961) and Marumaya (1968) both plastids and mitochondria differentiate to hardly recognisable forms, barely the expression of their genomes. Mitochondria undergo a phase of division and then dedifferentiate into small, spherical, condensed organelles (Fig. 8), measuring about 0.1 μm in maximum dimension. The extent to which plastids dedifferentiate depends upon the species, but in *Lilium* they are converted of featureless, pleomorphic inclusions (Figs 3-5) with little or no identifiable content (Dickinson, Heslop Harrison, 1970a, b). In *Tradescantia* (Marumaya, 1978) they may retain some starch. It is unlikely that starch is removed from these plastids for use in energy production, for concomitant with its disappearance from the plastids, large amounts of lipid appear in the cytoplasm in droplets (Figs 1, 3). Since the callose special wall around the protoplast is nearly impermeable (Heslop Harrison, Mackenzie, 1967), it is not unreasonable to propose that the precursors of this lipid are from the plastids.

It is not certain that all organelles survive this phase of reorganisation, for in many plants profiles are present indicating the demise of both plastids and mitochondria (Fig. 7) (Bell, Mühlethaler, 1964; Dickinson, Potter, 1978; Dickinson, Potter, 1979).

Recent information (Dickinson, Potter, 1973) has served to confirm that the changes overcoming both organelles and cytoplasmic RNA during male meiotic prophase, also occur in female cells. While the significance of these events is discussed later in a wider context, it is probably as well here to examine the nature of this cytoplasmic dediferentiation. If all species so far examined are taken into account, the data do not suggest that elimination of the preprophase ribosome population is the sole purpose of these events, although this has been proposed (Siersma, Chiang, 1971). Instead, the evidence points rather to the removal, maybe to elsewhere in the cell, of some other

All material depicted in these plates was prepared as described in Dickinson and Heslop Harrison (1970a).

Fig. 1. Tapetal cell (T) and young meiocyte (M) of *Lilium* in early prophase. Note the equal numbers of ribosomes between tapetum and meiocytes. Plastids (P) and mitochondria (H) are both visible in the meiocyte cytoplasm. × 14733

Fig. 2. Tapetal cell (T) and young meiocyte (M) of *Cosmos* in mid-prophase. Note the low ribosome numbers in the meiocyte. × 14020

Fig. 3. Dedifferentiation of plastids (P) and synthesis of lipid bodies (L) in the early prophase cytoplasm of *Lilium* meiocytes. × 13600

Fig. 4. Pleomorphic plastid in the cytoplasm of a *Lilium* meiocyte in mid-prophase. × 32650

Fig. 5. Dedifferentiated plastid (P) in the late prophase cytoplasm of *Lilium* male meiocytes. × 41020

Fig. 6. Pollen mother cell cytoplasm of *Lilium* at pachytene, showing small 'capsules' of cytoplasm (arrows) containing high concentrations of ribosomes. × 11180

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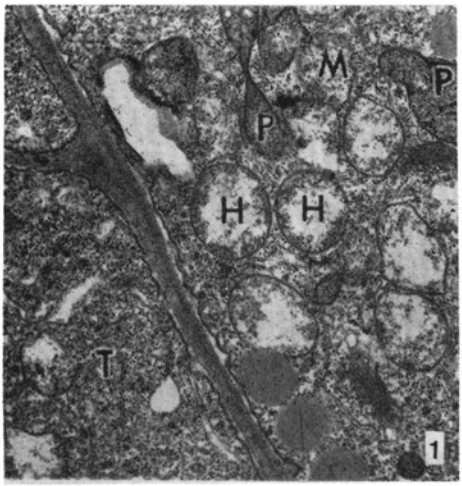
form of RNA. It is not impossible that this polynucleotide is so intimately bound to ribosomes in some plants that these organelles are degraded also. If this species of nucleic acid included long-term messenger RNA, dedifferentiation of both plastids and mitochondria would be explained, as would the infrequency of polyribosomes characteristic of this period (Linskens, Schrauwen, 1968).

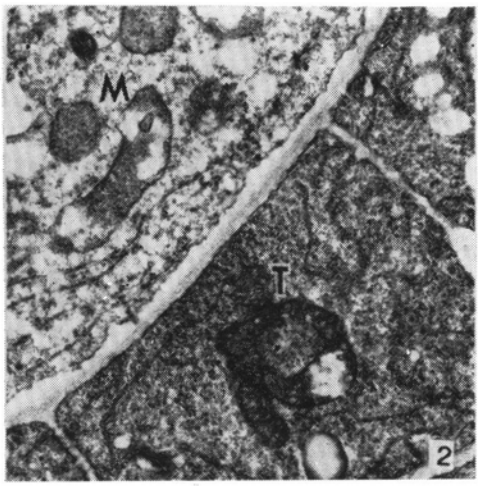
Some protein synthesis is undoubtedly required during meiotic prophase (Hotta, Stern, 1971) and this would clearly be difficult in a cytoplasm apparently denuded of ribosomes. However, in species where this purging of the cytoplasm is particularly thorough, there is evidence that a small percentage of the cytoplasm is encapsulated is small (Fig. 6) or multi-membraned inclusions (Rodkiewicz, Mikulska, 1965; Dickinson, Andrews, 1977) in which protein synthesis may continue unaffected.

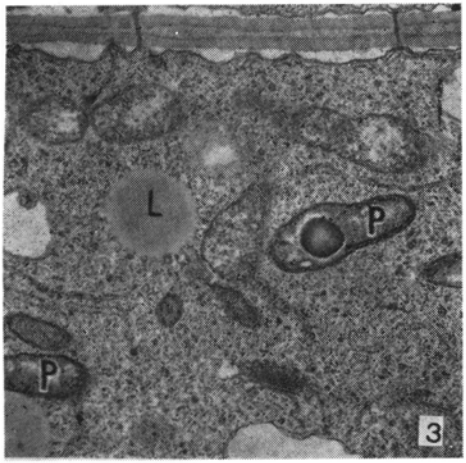
THE CYTOPLASM DURING AND SUBSEQUENT TO MEIOSIS

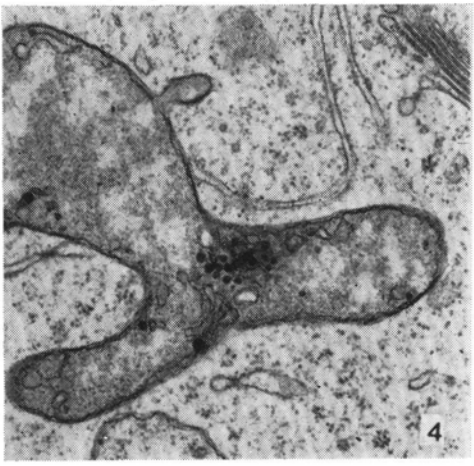
In all plants so far examined, maximum dedifferentiation of the cytoplasm has taken place by the pachytene stage of meiotic prophase. Little further change occurs until telophase I, when the cytosol begins to be repopulated with ribosomes (Dickinson, Heslop Harrison, 1970 b). Events of significance do however occur in the nucleus at this time, some apparently unconcerned with the meiotic process. Most conspicuous is an activation of the nucleolar organiser (Fig. 9), (Williams et al., 1973) which produces chains of small accessory nucleoli. These nucleoli subsequently detach themselves from the chains and float free in the nucleoplasm. Strikingly, they disappear during diplotene only to become evident just prior to metaphase I. Preliminary labelling experiments suggest that the nucleolar RNA becomes tem-

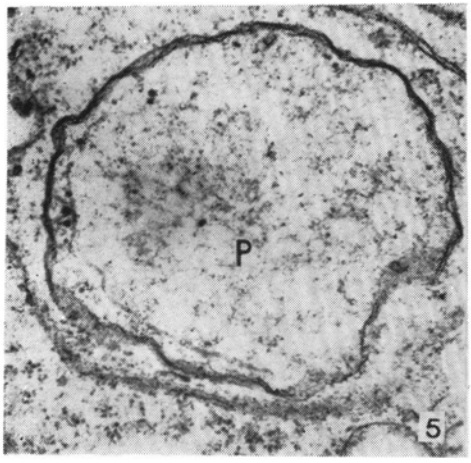
PLATE I

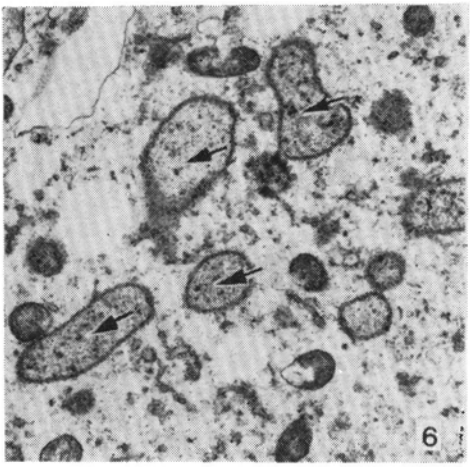












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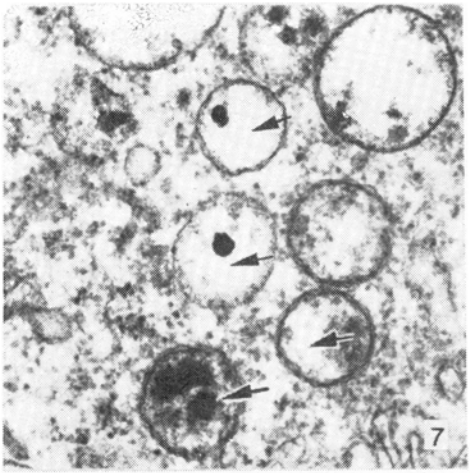

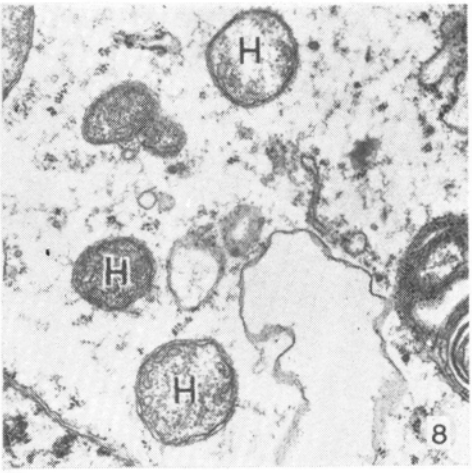

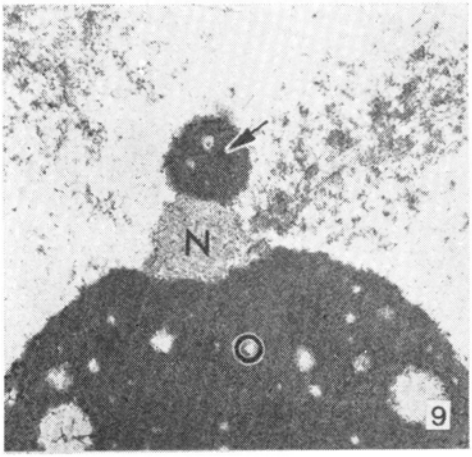

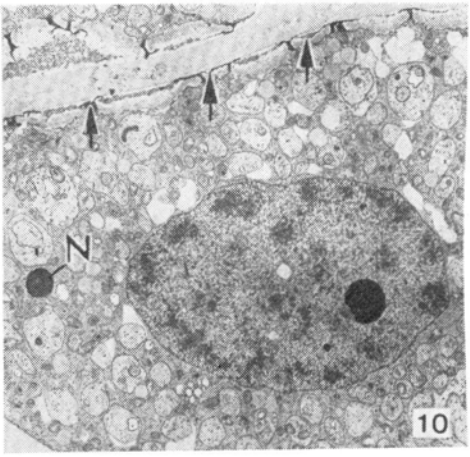

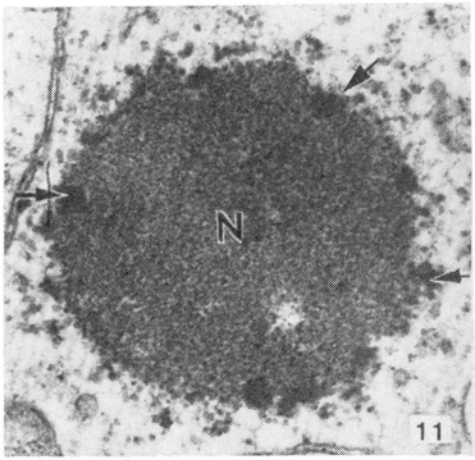

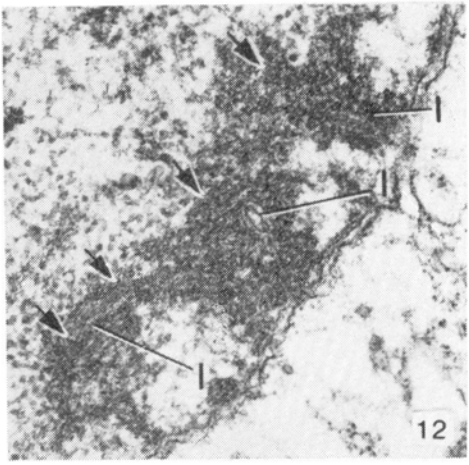

Explanation as in Plate I.

Fig. 7. Late prophase cytoplasm of *Cosmos* meiocytes. Note the presence of profiles (arrows) indicating degeneration of organelles. × 50610

Fig. 8. Small condensed mitochondria (H) in the meiotic cytoplasm of *Lilium*. × 24220

Fig. 9. Activation of nucleolar organiser (N) in cells of *Lilium* at the pachytene stage of meiosis. Note the mother cell nucleolus (O) and the presence of a new accessory nucleolus (arrow). × 21000

Fig. 10. Cytoplasmic nucleoloid (N) present in the cytoplasm of *Lilium* tetrad of young microspores. Note the formation of the primexine of the pollen wall (arrows). × 1746

Fig. 11. Disintegration of a cytoplasmic nucleoloid (N) in the tetrad cytoplasm of *Lilium*. Note the 'clumping' of ribosomes (arrows) around the edge of this aggregation. × 65680

Fig. 12. Nucleo-cytoplasmic interaction in the post-meiotic cytoplasm of *Pinus*. The nuclear envelope has formed invaginations (I) which are themselves invested in chromatin (arrows). × 29600

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porarily associated with the condensed chromatin. Since they form no connection with the spindle apparatus, at anaphase I they remain isolated at the metaphase plate and following formation of the dyad, become dispersed in the cytoplasm (Fig. 10). Here they progressively decrease in size becoming undetectable by the time the tetrad breaks up. Inspection with the electron microscope reveals that these organelles, termed cytoplasmic nucleoloids, fragment to form the new meiotic population of ribosomes. It is striking that as these 'new' ribosomes detach themselves from the mass of the nucleoloid (Fig. 11), many of them are in polyribosome configuration. This activity of the nucleolar organiser and the production of cytoplasmic nucleoloids appears to be correlated closely with the degree to which ribosomes become absent from the cytosol in early prophase. For example groups of species such as *Lilium*, *Rheo* and *Zea* produce large numbers of nucleoids, whereas *Pinus* in which the cytoplasmic RNA decline in prophase is really only detectable with the microdensitometer, contains only small aggregations of RNA in the post-meiotic cytoplasm. In some plants, this immediate post-meiotic period is also characterised by activity at the nuclear envelope. In gymnosperms, for example, nuclear pores become modified to form finger-like invaginations (Fig. 12) into the nucleoplasm (Aldrich, Vasil, 1970; Dickinson, Bell, 1970). These invaginations, which are formed by proliferation of the nuclear envelope, form swellings at their tips which become filled with electron-opaque fibrils. While these fibrils are accumulating, granular material amasses around the thin neck of the invagination. Enzymic digestion shows these granules to be rich in DNA, while the fibrils appear to consist solely of RNA (Dickinson, Potter, 1975). A single fibril extends from the swollen distal

Explanation as in Plate I.

Fig. 13. Interaction between nucleus (N) and mitochondria (arrows) in the tetrad of young microspores of *Cosmos*. × 5100

Fig. 14. Detail of material as shown in Fig. 13. Note the characteristic central vesicle (V) of the mitochondria, and the apposition of chromatin (C) subjacent to the organelle. × 107310

Fig. 15. Plastids (P) darkening in the cytoplasm of the young microspore of *Lilium*, now released from the tetrad. × 11400

Fig. 16. Redifferentiating mitochondria showing the appearance of electron- opaque material (arrows) in the matrix. Female material, but identical structures are present in male cells. × 16000

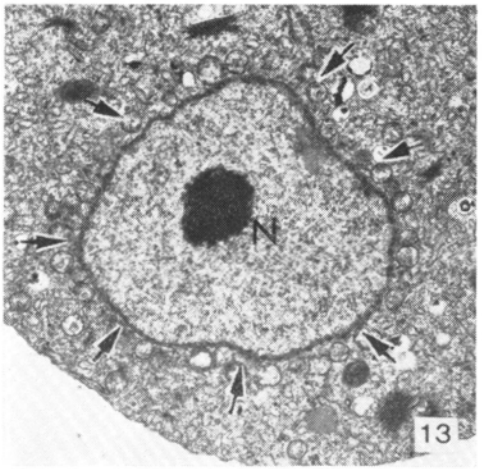
Fig. 17. The appearance of MPAs (arrows) in redifferentiating plastids (P) in the tetrad cytoplasm of *Lilium*. Mitochondria (M) containing electron-opaque material are also visible. × 16200

Fig. 18. Mature amyloplasts (A) in the young pollen grain of *Lilium*. Note the protein bodies (P) and early generative cell cytoplasm (G). × 7437

region of the invagination to the orifice, a modified nuclear pore, in the nuclear envelope. Polyribosomes are frequently observed associated with, or adjacent to these pores (Dickinson, Bell, 1972).

Formation of the tetrad also presages changes in the mitochondria and plastids. The mitochondria, while still in their condensed "promitochondrial" form first develop aggregations of electron-opaque material in their matrices, and then enlarge to form a more orthodox morphology (Fig. 16). During this process the dark-staining material becomes dispersed throughout the matrix (Dickinson, Potter, 1978). Recent evidence also points to a stage of physical association between mitochondria and the nuclear envelope. This phenomenon has only been examined exhaustively in *Cosmos bipinnatus*, but, here, small promitochondria, all containing a characteristic central vesicle become affixed to the nuclear envelope (Figs 13, 14). Strikingly, elements of chromatin are also seen apposed to the inner face of the nuclear envelope in these regions (Dickinson, Potter, 1979). As far as may be determined from the electron micrographs, the mitochondria subsequently detach and redevelop as previously described.

The redifferentiation of the plastid population is also unusual, particularly in *Lilium* where it has been studied in detail (Dickinson, Heslop Harrison, 1970a). Shortly after meiotic prophase, an ordered array of electron opaque particles becomes associated with a small piece of membrane in the plastid stroma. The whole region then darkens throughout meiosis II until by the tetrad stage it consists of a conspicuous dark patch, containing the membrane-particle association (MPA) (Fig. 17). It is interesting that at this stage, workers using permangante as fixative do not see these MPAs, but rather 'clear areas' containing the fibrils of plastid DNA. The whole stroma of the plastid darkens on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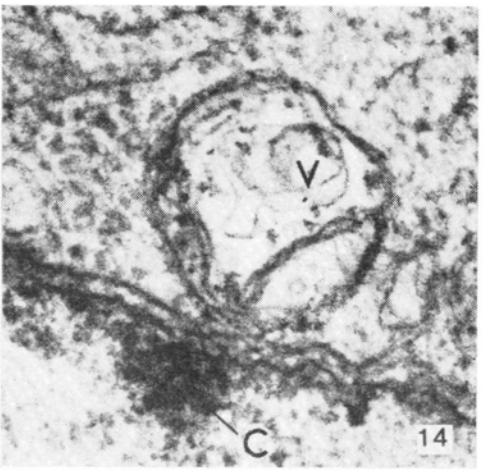
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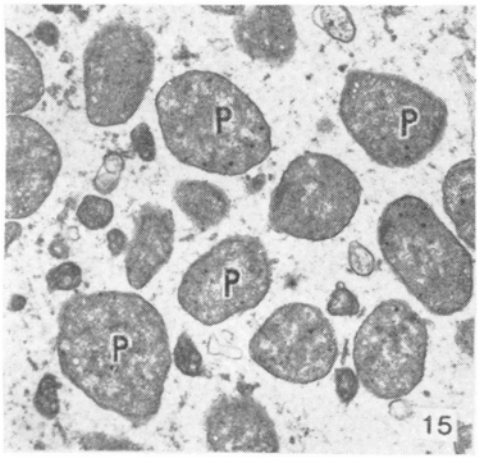
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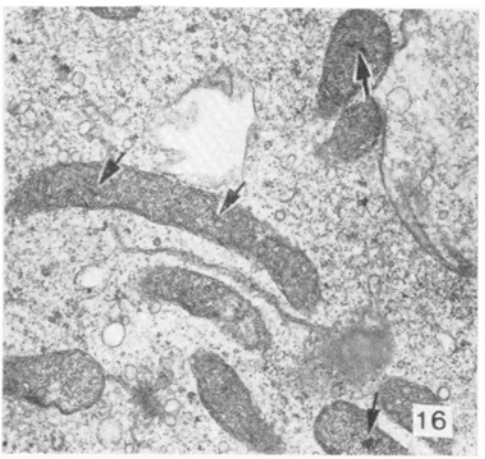
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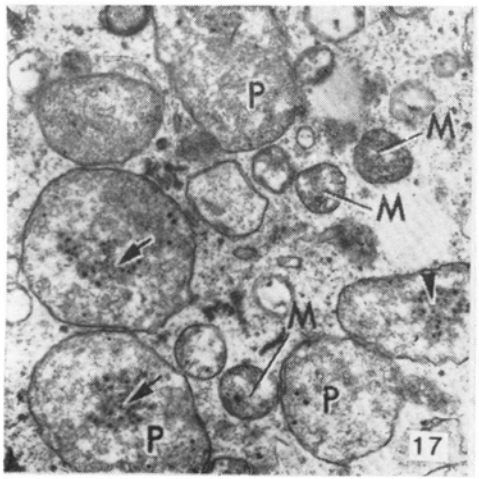
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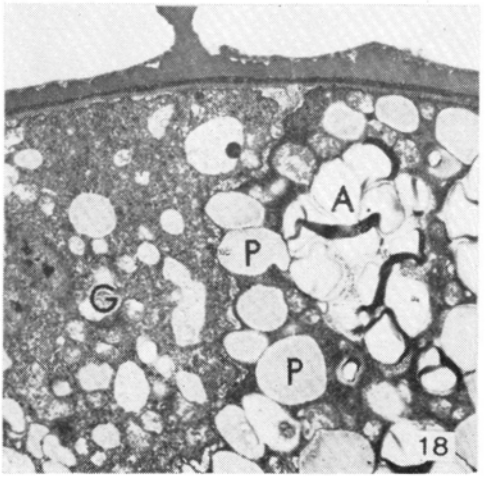
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rupture of the tetrad (Fig. 15), membrane proliferation occurs, and these proplastids differentiate into amyloplasts in a classical fashion (Fig. 18). This redifferentiation may be preceded by division of the proplastids and it is perhaps significant that, in this case, the MPAs divide in synchrony with the organelle.

The multimembraned inclusions (MMI) formed prior to meiotic prophase are degraded prior to the break up of the tetrad of microspores. Their content becomes granular, then diffuse and they finally rupture leaving sections of membrane, many of which appear to be involved in the formation of the nexine I layer of the pollen wall.

THE SIGNIFICANCE OF CYCLES OF CYTOPLASMIC DIFFERENTIATION DURING MICROSPOROGENESIS

From the preceding there is clearly much evidence of a cycle of cytoplasmic dedifferentiation and redifferentiation accompanying microsporogenesis in higher plants. The callosic wall investing the cells undergoing these changes is notoriously impermeable (Heslop Harrison, Mackenzie, 1967) and it is thus important to establish whether these events may be explained by starvation of the tissues. Four pieces of evidence suggest that they may not; firstly, an organised conversion of resources from starch to lipid takes place in prophase, lipid which remains present until the tetrad stage. Secondly, the wave of lytic enzyme activity that passes through the prophase cytoplasm indicates that the removal of much of the RNA is not a consequence of lack of reserves. Similarly the fact both plastids and mitochondria commence redifferentiation before rupture of the tetrad underlines the presence of an organised sequence of cytoplasmic differentiation and, fourthly, the isolation, in some species, of areas of cytoplasm by multimembraned inclusions rendering them impervious to the degrading enzymes of the cytosol cannot possibly be explained in terms of starvation.

Thus, since an organised cycle of differentiation does appear to accompany microsporogenesis in many plants, can it result from a single sequence of events with its primary effect on one component of the cell, or rather be due to many cycles of activity occurring in parallel? Certainly, in higher plants a definite cycle occurs in the levels of cytoplasmic RNA. Equally certainly, a cycle of dedifferentiation and redifferentiation takes place in the organelles of many of these species. Both these cycles may however, be explained if during the fall in RNA levels in prophase long term messenger RNA is removed, and new messenger RNA only restored to the cytoplasm in the tetrad. Since little transcription is likely to take place in the highly condensed chromosomes of meiosis the organelles, without any 'support' from the nuc-

leus, would in time dedifferentiate simply the experession of their own genomes.

There are good reasons why messenger RNA might be removed from the preprophase cytoplasm. Since we are dealing with events that are common to micro, and megasporogenesis, the cytoplasm that emerges from this cycle of RNA metabolism passes on, in the female case, to form the basis of the next generation. There must thus be great selective pressure to eradicate any errors in any postranscriptional control elements and presumably also any others of extracellular origin. Similarly, meiosis in higher plants is also the point at which generation alternates. Completely new sets of genes are activated and, again in higher plants, new genomes are formed. It is perhaps not therefore a coincidence that impermeable callose always separates sporophyte from gametophyte, for if the two cytoplasms came into contact (as may happen in some self-incompatibility systems) they might recognise each other as "non-self" (Roberts et al., 1980). For this reason it is important that the cytoplasm be cleared of all long-term control elements pertaining to the sporophyte generation. Finally development of the gametophyte must require a rapid and very different period of differentiation. A plant would be very well placed selectively were it able to cleanse its cytoplasm in preparation for this differentiation. A requirement for the removal of long-term messenger RNA would also explain the vast variability in the way ribosomes are affected. It may simply be that in some plants the control elements are so tightly bound to ribosomes that they also have to be degraded to ensure elimination of the element. Variation in the 'strength' of the agent responsible for the elimination of the RNA is of course, not impossible.

The fact that the RNA, once degraded, apparently passes to the nucleus (Mackenzie et al., 1967; Williams et al., 1973) raises a further, most interesting, possibility. It has been shown that when RNA is applied to chromosomes *in vitro* it can induce them to pair (Huskins, Chen, 1950) and, similarly, it is perhaps possible that the presence of the degraded RNA in the nucleus is associated with the meiotic process (Bell, P.R. pers. comm.). The fact that apospory in ferns is apparently not accompanied by a fall in ribosome number might be considered as supporting such an hypothesis (Bell, 1980). However, since ribosome number is frequently an inaccurate indicator of changes in the cytoplasmic RNA fractions (Siermsa, Chiang, 1971), and since changes in ribosome frequency are easily detected only in angiosperms (Dickinson, Bell, 1976), more conclusive evidence will perhaps come from apomictic cells of angiosperms.

There is little doubt that both the ribosomal and messenger RNA fractions are restored to the cytoplasm following meiosis. The ribosomes

return via the cytoplasmic nucleoloids so characteristic of the tetrad cytoplasm of many plants; it is, however, unlikely that they are already associated with messenger at this stage since following their formation at the nucleolar organiser, they have only been in contact with chromosomes involved in diplotene and diakinesis — chromosomes unlikely to be involved in transcription. In gymnosperms, on the other hand, the modifications of the nuclear envelope appear to be concerned with facilitating the transport of RNA to the cytoplasm, and its incorporation into polyribosomes (Dickinson, Bell, 1972).

While dedifferentiation of the organelle population would be expected to occur if long-term control elements were removed from the cytoplasm, and equally a transfer of energy reserves from an intra- to an extra--organellar form might be anticipated in these circumstances, why a large proportion of these organelles are eliminated is not known. There is evidence from recent work on *Saccharomycetes* that, in the course of sporulation, reassortment of mitochondrial genetic material takes place (Wilkie, 1973). While it is tempting to propose that equivalent processes might be taking place in both nucleus and organelle at this point, there is no equivocal evidence in support of such a conclusion. Without further data, it is also impossible to determine the significance of the association between the nucleus and mitochondria seen in the early tetrad. This phenomenon, which occurs in animals (Baker, Franchi, 1969) may simply represent structural evidence of the high energy requirement of the tetrad nucleus, or more interestingly, be part of a process by which information-carrying molecules are exchanged between nucleus and organelle. The striking redifferentiation of both plastids and mitochondria is most likely a result of starvation, in that the process may take place more slowly than usual. The dark material in the mitochondria has been identified with protein (Dickinson, Potter, 1979) whereas the MPAs of plastids appear to be a mixture of RNA and protein (Dickinson, Potter, unpublished), the appearance of which is entirely appropriate to the reactivation of the organelles in question.

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