Culmination in the slime mould *Dictyostelium discoideum* studied with a scanning electron microscope

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**SUMMARY**

Myxamoebae of *Dictyostelium discoideum* were allowed to develop on cellulose acetate filters, and specimens taken at various stages of fruiting body formation were prepared for study by scanning electron microscopy.

In the immature fruiting body where the mass of pre-spore cells has just been lifted off the substratum by the developing stalk, the pre-spore cells are irregular in shape and are similar in appearance to cells in aggregates at earlier stages of development. As the stalk lengthens, the pre-spore cells gradually separate from one another and become rounded and elongate, but mature spores are not visible until the fruiting body reaches its maximum height. It is concluded that, contrary to previous reports, spore maturation is a slow process and is not completed until the sorus becomes pigmented.

The mature stalk is surrounded by a smooth cellulose sheath but this does not envelop the cells of the basal disc, which remain discrete. The fruiting body is enclosed in a slime sheath and this may be important in holding together the mass of spores.

**INTRODUCTION**

The sequence of morphological changes occurring during development of the cellular slime mould *Dictyostelium discoideum* is well established (see Bonner, 1967). In the later stages (culmination), a fruiting body is formed from the slug-like migrating pseudoplasmodium. The aggregate becomes erect, and cells from the upper region move from the periphery towards the centre and down through the cell mass to form a stalk that lifts the residual mass off the substratum to form the aerial spore mass (sorus). This process has been described from studies with the light microscope (Bonner, 1944; Raper & Fennel, 1952; Farnsworth, 1973) whilst particular aspects of spore formation (Hohl & Hamamoto, 1969) and stalk formation (George, Hohl & Raper, 1972) have also been studied by transmission electron microscopy. Nevertheless, the timecourse of spore maturation has not been firmly established. Bonner (1944, 1967) has claimed that it occurs rapidly during the early stages of stalk elongation whilst Raper & Fennel (1952) have claimed that maturation takes several hours and is not

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complete until the fruiting body is almost at its maximum height. Further disagreement concerns whether maturation begins at the apex of the mass of pre-spore cells (Bonner, 1944; Raper & Fennel, 1952) or at the base (Farnsworth, 1973). The present study with the scanning electron microscope (SEM) was undertaken to try to resolve these points and to study further formation of the stalk and basal disc.

**MATERIALS AND METHODS**

*Culture of Dictyostelium discoideum*

Myxamoebae of strain NC-4 were grown in dual culture with *Aerobacter aerogenes* on agar plates as described by Sussman (1966). To induce development, 0.05 ml of a suspension of washed myxamoebae (5 × 10^7 per ml) was placed on a 12 mm disc punched from cellulose acetate filter (Oxoid Courtaulds Ltd, Coventry) and incubated at 22 °C (Sussman, 1966).

*Preparation for SEM*

Aggregates attached to the discs were fixed with acrolein vapour and glutaraldehyde and were infiltrated with polyethylene glycol (P.E.G.: mol. wt. 600) during dehydration (Treffry & Watts, 1974). The infiltrated specimens were coated with palladium–gold shortly before examination in a Cambridge S4 Stereoscan Scanning Electron Microscope.

Some specimens were examined whilst frozen and without metallic coating using a method adapted from that described by Turner & Smith (1974). *D. discoideum* was allowed to develop on 12 mm cellulose acetate discs pre-coated with palladium–gold. The course of development was not affected by the coating. The discs were attached to specimen studs with a bezel, frozen in Freon 22 (quenched in liquid nitrogen) and stored under liquid nitrogen prior to examination.

The specimen studs were made of brass (12.4 mm diameter, 5 mm thick) and were fitted to a nylon shaft. A thin copper wire, soldered to the brass and running down the nylon shaft, earthed the specimen when in the SEM. Specimens could be examined in the SEM for up to 30 min without thawing.

**RESULTS**

Following cessation of migration, the pseudoplasmodium passes rapidly through a number of morphological stages to give the erect structure (second finger) shown in Fig. 1. The upper region is derived from the anterior of the migrating pseudoplasmodium and contains the pre-stalk cells, whilst the lower part is from the posterior of the migrating pseudoplasmodium and contains the pre-spore cells (Raper, 1940a). Examination of the surface of the second finger does not, however, indicate any demarcation between the pre-spore and pre-stalk regions. Most of the surface comprises polyhedral cells which can be
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distinguished since they seem to have raised cell margins. There is also a distinct apical tip and, even at high magnification, the tip appears perfectly smooth and little cell detail can be distinguished. The surface morphology of the second finger is thus quite similar to that of the erect first-finger stage (Treffry & Watts, 1974, 1976) that is formed some 6–8 h earlier, at the completion of cell aggregation.

Formation of the stalk

As the mass of pre-spore cells moves upwards, the base of the second finger becomes constricted and the stalk becomes visible (Fig. 2). The surface of the stalk is fairly smooth but there are clearly longitudinal elements that twist around the stalk and which may indicate the directions in which the cellulose fibres of the stalk sheath have been laid down.

Although the P.E.G. infiltration technique can be used successfully to preserve the short, thick stalks of immature fruiting bodies (Fig. 2B), it is less successful at preserving the stalks of mature fruiting bodies. These long stalks bend downwards and may be seen (Fig. 3A) to be shrunken and collapsed presumably because P.E.G. fails to replace cell water and to maintain cell turgor during dehydration. This indicates that the turgor pressure of the contents of the now highly vacuolated cells (George et al. 1972) is as important as the cellulose cell walls and the cellulose stalk sheath in maintaining stalk structure and function. Fig. 3B shows the appearance of the intact, mature stalk viewed when frozen and it may be seen that the surface of the cellulose sheath round the stalk is now quite smooth.

The rather irregular internal arrangements of cells in the stalk is shown in Fig. 4 where the stalk has broken close to the holdfast. The cells have thick walls and it is clear that there is a sheath round the stalk which remains separate from the walls of the outermost stalk cells. This is consistent with the conclusion (George et al. 1972) that the cellulose stalk sheath is secreted first and that only later do cells within the sheath secrete their own cellulose cell walls.

The stalk rises from a distinct basal disc which comprises cells derived from the posterior of the migrating pseudoplasmodium in contra-distinction to the stalk cells which are from the anterior of the pseudoplasmodium (Bonner, 1944). Fig. 5 emphasizes that the cells of the basal disc remain separate from those of the stalk. The cellulose stalk sheath does not extend to surround the basal disc but instead penetrates into the centre of the mass of basal disc cells. Some of the basal disc cells are obscured by the remains of the enveloping slime sheath. Around the basal disc there are cells similar in appearance to those of the basal disc but which are not attached to it. Presumably these are cells which failed to rise up with the mass of pre-spore cells and which were instead left on the substratum.
Formation of the spores

When the developing fruiting body first has a distinct stalk, there is still little detectable differentiation in the sorus, where the surface appearance resembles that of the second finger. There is a distinct apical tip which, even at high magnification, appears featureless, whilst the remaining surface of the sorus comprises rather ill-defined polyhedral cells. The upper part of a sorus at this stage is shown in Fig. 6 and is of the immature fruiting body in Fig. 2A. The apical region is surrounded by a depression or, in some specimens, a distinct groove. This feature could be explained if it were the region where cells move inwards to join the developing stalk, but it is believed that this occurs in the upper part of the apical papilla (Bonner, 1967). Freeze-dried specimens tend to fracture in this region, and it is probably a region of stress at the boundary between the pre-spore cells and the developing stalk.

Later, the outline of the pre-spore cells becomes more distinct since the cells, though retaining a polyhedral shape, begin to separate from one another (Fig. 7). The boundary between the pre-stalk and pre-spore cells is now very sharp. By the time the fruiting body approaches maximal height, the pre-spore cells have become smaller and elongate and have further separated from each other (Fig. 8). Hohl & Hamamoto (1969) have suggested that the cells decrease in size because of ejection of the contents of their pre-spore vacuoles into the intercellular spaces, so that the cells become embedded in slime. This intercellular material is evident in Fig. 8. In Fig. 9, a similar specimen, prepared by freeze-drying, is shown. The cells have collapsed, and this establishes that, at this stage the pre-spore cells still lack rigid cell walls.

Although the fruiting body in Fig. 8 was close to its maximal height, it is clear that spore maturation was not complete, particularly if Fig. 8 is compared with Fig. 10, which shows the sorus of a later fruiting body where the spores have attained a mature appearance. In Fig. 10 the cells are completely rounded and elongate and there is little cell–cell contact (Fig. 11a) at least at the surface of the sorus, owing to the random arrangement of the spores. The spores may be held together to form the sorus by intercellular slime, as suggested by Hohl &

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**Figures 1-4**

- Fig. 1. The second finger stage showing a clearly defined apical tip. P.E.G. infiltrated specimen.
- Fig. 2. (A) An early fruiting body. The pre-spore cells have lifted completely free of the substratum to reveal the lower part of the developing stalk. (B) Detail of stalk. P.E.G. infiltrated specimen.
- Fig. 3. Detail of the stalk of a mature fruiting body. Considerable shrinkage is apparent in the P.E.G. infiltrated specimen (A) but not in the frozen specimen (B).
- Fig. 4. Internal arrangement of cells in the stalk of a mature fruiting body. The stalk has fractured close to the basal disc, which is seen in the lower part of the micrograph. The stalk sheath (arrows) can be distinguished. Frozen specimen.
Hamamoto (1969), but the slime sheath that has enveloped the whole cell mass since aggregation (Treffry & Watts, 1975) may also be important, particularly in holding together the surface layer of spores. This sheath would be expected to collapse into an extremely thin film that would not obscure detail in dehydrated specimens examined at low magnification (e.g. Figs. 6–10) but it was visible in all frozen (non-dehydrated) specimens examined (e.g. Fig. 12). The collapsed sheath was also detected in dehydrated specimens examined at high magnification (e.g. the spores in Fig. 11a) where it obscured cell detail. Surface detail of free spores (Fig. 11b) at the same magnification is noticeably sharper.

DISCUSSION

In previous studies of culmination (Bonner, 1944; Raper & Fennel, 1952; Farnsworth, 1973), the course of development of individual aggregates was followed by time-lapse photography using a light microscope. Thus the time-course of development could definitely be established, but cell detail could not be distinguished in the developing aggregates because of the limited resolution of the light microscope. On the other hand, cell detail was easily resolved in the present SEM study but a single aggregate could not be followed throughout development, and the sequence of the stages of development could only be derived from the time sequence in which specimens were fixed for examination by SEM.

Differentiation of the stalk and basal disc

Development in Dictyostelium discoideum seems often to be considered in terms of differentiation of two types of cell (stalk cells and spores), the cells of the basal disc being regarded as the same as stalk cells on the basis of their ultimately similar ultrastructures (George et al. 1972). However, the SEM photomicrographs emphasize that the basal disc cells remain free of the stalk sheath and thus distinct from the stalk. The region of origin of the basal disc cells in the pseudoplasmodium is different from that of the stalk cells (Bonner, 1944) and, since the basal disc cells also undergo changes in ultrastructure on a

FIGURES 5–8

Fig. 5. Part of the basal disc of a mature fruiting body showing basal disc cells (b) overlying the lower region of the stalk sheath. Remains of the slime sheath (s) are visible. Frozen specimen.

Fig. 6. Upper part of the sorus of the early fruiting body shown in Fig. 2(A). The apical papilla is visible in the upper part of the photomicrograph and is surrounded by a depression (arrows) that distinguishes it from the mass of pre-spore cells. P.E.G. infiltrated specimen.

Fig. 7. A later stage in the development of the sorus. The individual pre-spore cells are clearly defined, but no cell detail is visible in the apical papilla in the upper part of the photomicrograph. P.E.G. infiltrated specimen.

Fig. 8. Sorus of an almost mature fruiting body. Part of the stalk may be seen (lower left). P.E.G. infiltrated specimen.
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much longer time scale than cells at the base of the stalk (George et al. 1972), it may seem more reasonable to consider the cells of the basal disc as a third group of cells produced, along with the stalk cells and spores, by differentiation in *D. discoideum*.

**Maturation of the spores**

There has been some disagreement concerning the time course of spore maturation. Farnsworth (1973) has presented a scheme for culmination in which mature spores are present at the periphery of aggregates as early as the second-finger stage, whilst Bonner (1944, 1967) has claimed that differentiation is later and takes place rapidly in about 30 min at the stage when the stalk has become approximately twice the length of the sorus. Raper & Fennel (1952) have suggested that a much longer period is required, the process not being completed until shortly before the fruiting body reaches its maximum height. Our studies are not consistent with the views of Farnsworth (1973) or Bonner (1944, 1967) – there being no spores visible at the second-finger stage (Fig. 1) or in early fruiting bodies (Figs. 6, 7) – but are broadly in agreement with those of Raper & Fennel (1952). It was difficult to determine when the fruiting bodies reached their maximum heights but it was probably about the stage shown in Fig. 8 where spore maturation was clearly incomplete. It is possible that Raper & Fennel (1952) may have mistaken cells at this stage for mature spores because of the limited resolution of the light microscope and so even they may have underestimated the time required for spore maturation. We have not detected mature spores at the surface of the sorus until the period when the sorus becomes pigmented, which is between 24 and 30 h development on Millipore filters in the conditions described by Sussman (1966).

Raper (1940b), Bonner (1944) and Raper & Fennel (1952) have also concluded that spore maturation begins at the surface of the sorus and proceeds most rapidly near the apex, but it seems an essential feature of the account of fruiting body development given by Farnsworth (1973) that spore maturation

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**Figures 9-12**

Fig. 9. A specimen similar to that in Fig. 8 but prepared by freeze-drying. The pre-sporocyte cells have collapsed, indicating the absence of rigid cell walls.

Fig. 10. Mid-region of the sorus of a mature fruiting body. The spores appear to be arranged randomly. P.E.G. infiltrated specimen.

Fig. 11. (A) Mature spores. There is little contact between spores. P.E.G. infiltrated specimen. (B) Mature spores detached from a damaged fruiting body. Cell detail is clearer than in (A) since the spores are not covered by the remains of the dehydrated slime sheath. P.E.G. infiltrated specimen.

Fig. 12. Sorus of a mature fruiting body. The specimen was prepared by freezing and this has preserved the slime sheath (s) surrounding the sorus. Spores are visible in the upper part of the photomicrograph where the sheath has split during preparation of the specimen.
should be most rapid at the base of the sorus. However, the surface cells of the sorus always appeared uniform on careful examination of any developmental stage with the SEM and no differences have been detected in the progress of maturation in the upper and lower regions of developing sori.

Formation of the fruiting body

Considerable movement and rearrangement of cells is required during culmination. Raper & Fennel (1952) have provided the only account that attempts to explain in detail how this occurs. However, their account now requires some modification as a result of the transmission electron microscope studies of George et al. (1972) and the present SEM studies.

At the second-finger stage, a central core of pre-stalk cells is present surrounded by a cellulose sheath which may have been secreted by the cells enclosed within it, though this has not been firmly established. However, extension of the sheath during elongation of the developing stalk takes place only at the apical tip where pre-stalk cells secrete the cellulose (George et al. 1972) as they move upwards along the outside of the sheath. Finally, these cells move into the extended sheath, where they secrete their own cellulose cell walls and differentiate into stalk cells. The account given by Raper & Fennel (1952) implies that only the surface cells at the top of the apical papilla can move into the extended sheath, but we have been unable to observe with the SEM the invagination that would be expected to result from such movement of surface cells, and it would seem that it is cells lying somewhat deeper in the papilla that are involved in the movement.

Since the sheath is extended by addition of cellulose at its tip rather than at its base, the pre-spore cells cannot be carried passively into the air but must move up the stalk sheath. Possibly this is by amoeboid movement (Raper & Fennel, 1952) since this is the only means of movement used by the other stages in the life-cycle of *D. discoideum*, and amoeboid movement of cells along the stalk has been observed in other species of *Dictyostelium* (Bonner, 1967). The discovery, using the SEM, that the prespore cells do not mature into spores until the stalk approaches its maximum height is thus of considerable significance, since it is compatible with the suggestion that the cells in the ascending pre-spore mass are capable of amoeboid movement. Certainly there seems to be no way of explaining how mature spores could ascend the stalk if, as has been previously claimed, they are formed from the pre-spore cells long before the stalk is completed.
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REFERENCES


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