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Some Observations of Ascospores of *Neurospora crassa* Made with a Scanning Electron Microscope

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Scanning electron micrographs of ascospores of *Neurospora crassa* reveal two of the structures which develop during germination and outgrowth: (i) a germination pore and (ii) the probable site of initiation of hyphal cell wall synthesis.

Observation of gross morphological changes is an important prerequisite to a study of biochemical events in a developmental sequence. The scanning electron microscope (SEM) is used for this purpose with microorganisms since surface features and the three-dimensional arrangement of parts are easily seen with this instrument.

Accordingly, we present preliminary observations of the ascospore of *Neurospora crassa* during the transition from spore to vegetative state. The germination and outgrowth of ascospores of this organism constitute one of the simplest developmental programs to be found in a eukaryotic system. This process has been examined with the transmission electron microscope in the closely related species *N. tetrasperma* (1). To our knowledge, there has been only one other SEM study of the germination and outgrowth of a eukaryotic spore (2), that of *S. cerevisiae*, and only one other SEM study of *N. crassa* (3).

We present here micrographs of two of the structures which develop during the germination and outgrowth of the ascospore of *N. crassa*, namely, (i) a distinct germination pore which was visible only in spores which had undergone heat activation and (ii) the probable site of initiation of hyphal cell wall synthesis.

Ascospores were obtained from the cross 74a × 74A in petri dishes (100 by 15 mm) on Westergaard's synthetic cross medium (5) with 2.0% sucrose. Activation was accomplished by incubation in water at 60 C for 45 min. Spores were then incubated in Vogel's medium N (4) on a reciprocal shaker at 35 C. Germ tubes were visible after 2 to 3 hr under these conditions.

Fixation was in absolute ethanol-acetic acid (3:1) for 1 hr. Spores were then washed once with distilled water, filtered onto nitrocellulose filters (0.8-μm pore size, Millipore Corp.), and air dried. Filters were mounted onto SEM stubs, coated with gold-palladium (60:40) in a Denton DV 502 vacuum evaporator, and viewed with a Cambridge Stereoscan Mark 2A scanning electron microscope. (All figures present spores fixed in absolute ethanol-acetic acid.)

Ascospores allowed to germinate directly on nitrocellulose filters fixed with 1.0% glutaraldehyde, washed, and air dried were also examined, with completely similar results. Germ tubes in this preparation had a slightly smoother surface.

Figure 1a is a scanning electron micrograph of an activated ascospore of *N. crassa*. The germination pore visible at the end of the spore was not found in any spores which had not undergone activation. Unactivated spores were either smooth at the end or slightly indented. A similar germination pore is visible at both ends of the ascospore of *N. tetrasperma*, both before and after germination. In one of the two spores shown in Figure 1d, a pore is clearly shown at the end at which no outgrowth occurs. Therefore, presumably in *N. crassa* germination pores are present at both ends as is the case in *N. tetrasperma*. The ribbed surface for which the genus is named is clearly visible.

Figures 1b and c are two magnifications of a spore at an intermediate stage of outgrowth. The structure in Fig. 1c between the spore and the hypha is apparently part of the protoplast. Structures similar to this were frequently seen in both preparations and probably result from the protoplasts being pulled from the spore

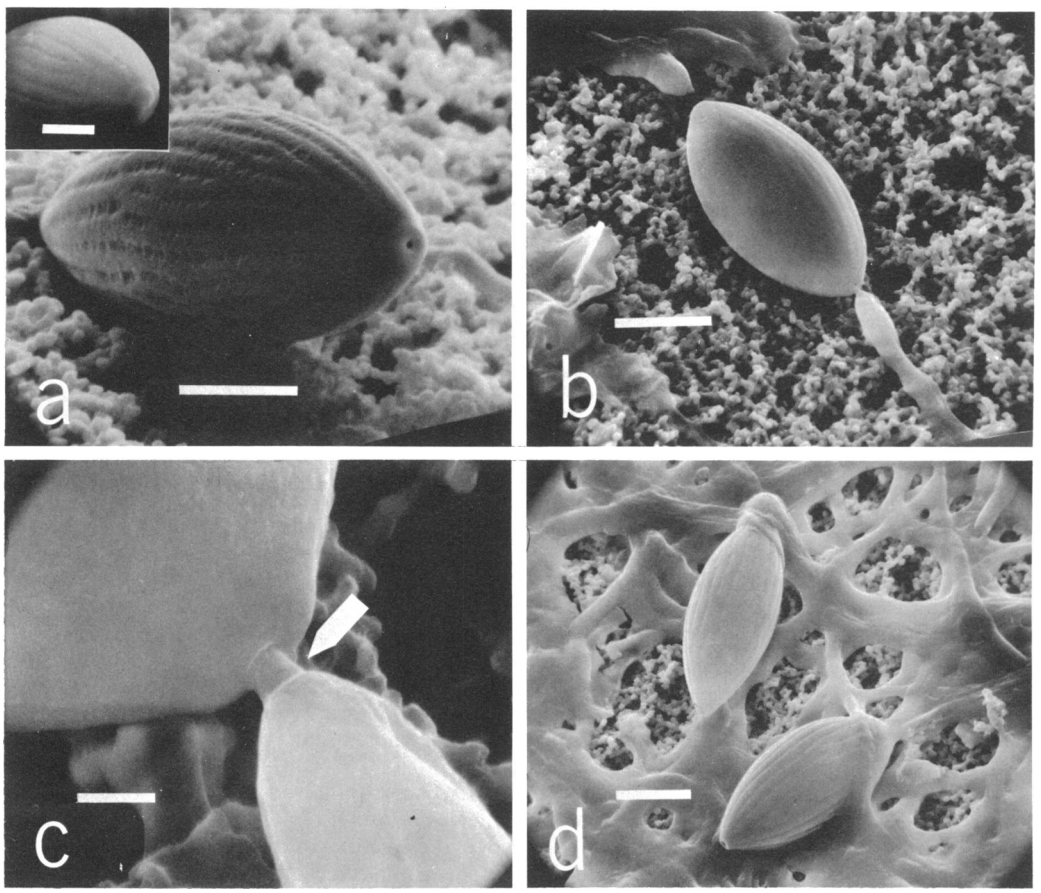

FIG. 1. *Scanning electron micrographs of ascospores of N. crassa during germination and outgrowth. Background material is nitrocellulose filter. a, Activated spore (an unactivated spore is shown in the insert); b, intermediate stage of outgrowth; c, detail of b (arrow indicates probable site of initiation of cell wall synthesis); d, advanced stage of outgrowth. Bar in a, b, and d represents 10 μm; in c, 1.0 μm.*

case. We interpret the rim visible on the hyphal side of the structure as the site of initiation of cell wall synthesis.

Figure 1d is a pair of spores at an advanced stage of outgrowth. The mycelial mat is only in part a result of the outgrowth of these two spores. Extensive fusion of the spore case and the growing mycelium occurs at this stage. The spore case is presumably digested.

Changes in the ascospore surface similar to those in *S. cerevisiae* were not seen at any stage.

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