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Procedure for preparing individual asci of Neurospora for electron microscopy

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Abstract

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Basl, M. and A. M. Srb. Procedure for preparing individual asci of Neurospora for electron microscopy.

The following procedure has been used with repeated success for ultrastructural studies of developing asci of N. crassa and N. tetrasperma. The procedure provides excellent material for ultrastructural work and permits the investigator to focus efforts on particular asci at a specific developmental stage. The procedure also provides a basis for serial sectioning of identified asci. Perithecia containing asci at the desired stage of development are scraped from the surface of petri plates containing crossing media (Westergaard and Mitchell, 1974, Amer. J. Bot. 34: 573) and transferred to a vial containing Karnovsky's (1965, J. Cell Biol. 27: 137A) cacodylate-buffered 3% formaldehyde-3% glutaraldehyde + 10% sucrose (w/v) adjusted to pH 6.8. Allow the preparation to stand 2 hr. at room temperature; then using an eyedropper transfer numerous perithecia (about 1/2 eyedropperful) onto the center of an inverted lid of a plastic Petri dish (30 x 150 mm). The bottom of the Petri dish is then fitted inside the lid so as to form a press. Using pressure, combined with a rotating motion, crush the perithecia, thereby liberating asci. Remove the bottom, hold the lid at an angle and flush the area with an eyedropperful of fresh fixative. Pick up the resulting material with the eyedropper and deliver into a double layered cheesecloth filter formed over a 15 ml centrifuge tube kept on ice. Repeat this procedure until all of the perithecia have been transferred. Concentrate the sample by centrifugation, if necessary. The filtrate and consequently the pellet contain some perithecial and hyphal debris, which is, however, easily recongized in the final preparation and does not present problems. Further steps are carried out in the cold (ice bucket or refrigerator) until noted otherwise.

After an additional hour in fixative, pellet the asci by centrifugation at 200 g for 4 min. and decant the supernatant. Suspend the asci in a 0.1 M cacodylate buffer pH 6.8 + 10% sucrose; after 20 min., centrifuge as before. Repeat rinsing procedure 4 times more. Decant supernatant after the 5th rinse, suspend the pellet in 2% OsO_4 in 0.1 M cacodylate and refrigerate overnight. Rinse the sample 5x (10 min. each) with 0.1 M cacodylate followed by 2 quick rinses in water. Dehydrate (3 min. each) in a graded acetone series (possible because asci pellet readily once out of 10% sucrose) of 25%, 50%, 75%, 2 changes in 90%, (taking to room temperature at the first 90% step) and 3 changes in 100% acetone. After decanting the supernatant at the final 100% acetone step, add 2 drops of epon-araldite mixture (Mollenhauer, 1964, Stain Tech. 39: 111) to the pellet covered by a small amount of acetone that inadvertently remains. Suspend the pellet in the acetone-plastic by stirring gently with an applicator stick. After 1 hr. add freshly prepared epon-araldite (ca. 3 ml is usually sufficient, but the amount depends on size of the sample), resuspend by mixing as before, pick up entire sample with a pasteur pipet and dispense 3-4 drops each in aluminum weighing dishes. Using a tongue depressor, gently spread sample over the mid-portion of the weighing dish so that a thin sheet results. Take care not to cause marks on the bottom of the dish. Place the sample in a 70° C oven after an additional 3 hr. at room temperature and leave overnight. Remove the cooled plastic sheet from the weighing dish by turning the edges of the dish down and gently bending the bottom downward. The sheet will peel off.

Prepare the embedded asci for examination with the phase contrast microscope by first trimming away the edges of the plastic sheet with a sharp razor blade. Then with the razor, cut loose a section (10-15 mm square) and with a drop of clear fingernail polish attach it bottom side up to a glass slide. Because the asci tend to sink in unpolymerized plastic, they are more readily available for observation if mounted bottom side up. Place the slide with the mounted plastic in a 70° oven for 1 hr. or longer. The asci can now be examined with a phase contrast microscope. The asci lying closest to the plastic surface can be observed with the oil immersion objective. (Cytological observations here are more difficult than with material prepared specifically for conventional microscopy. However, nuclear areas are distinguishable in that they appear smooth in contrast to the granular appearing cytoplasm. In uninucleate asci nuclear areas are readily observed, but are increasingly difficult to discern as the nuclear divisions progress. Fortunately, other features aid in determination of a developmental stage, which later can be verified by electron microscopy. Different strains provide different landmarks, which once determined are reliable. E.g., wild type strain N. tetrasperma (T-220) accumulates vacuoles in the mid-region of its cytoplasm during the interphase following meiosis. When vacuoles of unique appearance are seen in this region the nuclei are in mitotic metaphase.)

Select a desired cell, note its orientation and relocate it with the aid of dissection microscope and substage lighting. Wipe away the oil and with a razor blade cut out a small block containing the desired cell. The plastic readily snaps free from the slide despite the fingernail polish and care must be taken that the area of desired plastic is not lost. Loss can be minimized by applying slight pressure with fine forceps (Dumont #5) on the area to be saved while doing the final trimming. Place the trimmed cell in a small drop of Scotch brand eposy resin on another glass slide. Using a toothpick apply epoxy resin to the top of a blank mount [prepared by polymerizing Spurr's (1969, J. Ultrastr. Res. 26: 31) epon in 00 beem capsules] and place the trimmed block atop. Check with the aid of a dissection microscope to assure that the bottom side of the plastic is the same as when mounted to the glass slide and that it is properly centered on the blank. Insert the mounted blank, bottom end first, into an empty beem capsule so that the mounted end is sticking up. Place the "loaded" capsule into a beem capsule holder and place in a 70° oven overnight.

The specimen is readied for thin sectioning by first manually trimming it to as small a block size as possible. Then 1-0.5 μ sections are taken on an ultramicrotome using a glass knife. Watch for any subtle irregularities in the plastic that may serve as an indicator that the cell is present. When seen, mount that section on a slide, stain with azure B and ascertain the presence of the cell. When the cell is found, thin section the remainder of the cell with a glass or, preferably, a diamond knife. Pick up sections on formvar-coated grids and stain with uranyl acetate and lead citrate before viewing with a transmission electron microscope 60 KV.

This procedure was originally done with Spurr's epon rather than epon-araldite as the embedding plastic, but lack of contrast prevented good imaging at the EM level. (Supported by Grant GM-12953 from the National Institute of General Medical Sciences, U.S.P.H.S.) - - - Section of Botany, Genetics and Development, Cornell University, Ithaca, New York 14853.