How to use hematoxylin for cytological studies.

Background

McClintock, Singleton, and Barry found aceto-orcein excellent for visualizing fine structure of chromosomes at pachytene and other stages (see Barry 1966, 1996). In their squash preparations, however, the cytoplasm was characteristically filled with coarse 'bubbles' that often obscured observations. Lu and Raju (1970) developed a procedure using hemotoxylin (adapted from Henderson) which stains fungal chromosomes, nucleolus and spindle-pole bodies effectively while leaving the cytoplasm clear and transparent. (Compare photographs in Singleton 1953 and Raju 1980.) Aceto-orcein may still be preferred for fine details of pachytene chromosome morphology, however.

Procedure for examining meiosis and ascus development (From Raju and Newmeyer 1977)

Preparation of mordant and stain. A supersaturated mordant solution is made by adding 10 g of ferric acetate to 100 ml of 50% aqueous propionic acid in a glass-stoppered bottle. The bottle is stored at room temperature (~22º C) and occasionally shaken. After about 2 months, small amounts of the supernatant can be decanted to a dropping bottle for use, leaving the rest of the contents for further saturation. The mordant keeps well for several years at room temperature. The decanted mordant can be diluted with a few drops of 50% propionic acid if necessary.

A 2% solution of hematoxylin is prepared in 50% propionic acid and allowed to 'ripen' at room temperature for about a month in a half-filled glass-stoppered bottle. The ripened solution keeps well for several months at room temperature and may be stored at 4º C for 2 or more years.

Fixation. For studying meiosis and ascus development in Neurospora, crosses are made in petri dishes at 25º C on synthetic crossing medium poured to a depth of 2 or 3 mm. The protoperithecial parent is incubated for 5 days before fertilization. Strips of agar medium bearing perithecia are cut at intervals beginning 3 days after fertilization, the time when karyogamy occurs in the croziers. Pachytene and other early meiotic events are found in 4-day-old perithecia. Five to six-day-old perithecia are suitable for later stages of meiosis and spore delimitation. While 5- or 6-day-old perithecia contain asci in all stages of meiotic development, it is advisable to fix perithecia at 12-hour intervals between 3 and 8 days so that samples can be obtained that are optimal for specific stages.

The ~ 5 × 20 mm strips bearing perithecia are fixed in a freshly prepared mixture containing 9:6:2 absolute ethanol, propionic acid, and 10% aqueous chromic acid, using 15 ml screw-cap vials which are stored at -20º or 4º C. Recently fixed material gives superior preparations. Storage for periods longer than 1 month should be avoided.

Hydrolysis and washing. The hydrolyzing solution containing 1 : 1 concentrated HCl and absolute ethanol in a 12 × 100 mm glass tube, is heated to 70º C in a block heater or water bath. Fixed perithecia, still on the agar block, are plunged into this solution and allowed to hydrolyze for 1 min. After removal from the heater hydrolysis continues for 2 min, during which the solution cools to 45º C. Then the agar block is quickly transferred to a solution containing ethanol, acetic acid, and chloroform (3:1:1) (Carnoy solution). This removes HCl, which would interfere with staining. The chloroform also prevents vacuolization of the cytoplasm. Six- to eight-hour soaking in the Carnoy solution renders ascus cytoplasm less “bubbly”, perhaps by dissolving lipid vesicles, thus allowing the cell organelles to show up more prominently (see Raju 1978, 1980 for photographs).

Staining. Five to 10 perithecia are dissected in a small drop of ferric acetate solution on a microscope slide, using nichel-chrome needles to squeeze out the rosettes of asci. The rosettes are grouped, perithecial walls and other debris are removed, and excess mordant is blotted out. Then one drop of well-ripened

hematoxylin solution is added over the mordanted rosettes. These are immediately stirred thoroughly into the stain solution, using the flat end of a brass or glass rod. The preparation is then squashed under a loosely held cover glass by gently tapping directly above the rosettes to spread and break the asci. After excess stain has been pressed out, the cover glass is sealed with dental wax (Kerr's sticky wax).

The proportion of ferric ion and hematoxylin is very critical. Too much ferric ion precipitates hematoxylin, whereas insufficient ferric ion results in failure to stain chromosomes and spindle pole bodies. The staining is satisfactory when the stained rosettes of asci appear chocolate brown to the naked eye. Temporary cytological squashes remain good for about one week.

References

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