How to obtain naked nuclei extruded from the cell.

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Background

Naked nuclei extruded from conidia or other cell types are potentially useful for studies in cell biology.

Procedure

The following is copied from Raju (1998):

The silver-staining procedure described by Lu (1993) for surface-spreading synaptonemal complexes of Neurospora is also suitable for staining expelled paraphysal nuclei. Each nucleus shows a darkly-stained prominent nucleolus. I have adapted the staining method for routine observation of conidial nuclei. Conidia were fixed in a few drops of 4% paraformaldehyde for 3 or 4 min at 4ºC. A drop of conidial suspension was then placed on a glass slide and squashed under a cover glass with gentle blows from a rubber hammer to break open the conidia. The cover glass was lifted off after freezing the slide on a Cold Plate and the nuclei were flooded in a few drops of 4% paraformaldehyde + 0.04% SDS for 15 to 20 min. The slide was rinsed in distilled water, dipped in 0.2% Kodak Photoflo, and air-dried. For silver staining, one drop of 50% silver nitrate and one drop of gel developer (2% gelatin + 1% formic acid) were mixed together and added on to the slide and covered with a cover glass. The slide was heated on a hot plate at 45ºC for 2-3 min during which the nuclei were stained brownish to dark brown. The cover glass was promptly floated off and the slide was rinsed in distilled water and mounted under a new cover glass in a drop of 10% glycerol for microscopic examination. I find the silver staining particularly useful for examining nucleolus behavior in the segmental duplication strain *Dp(AR33)* that contains two normal nucleolus organizers (Perkins *et al*. 1980), and in the translocation strain *T(OY321)* that contains a split nucleolus organizer (Perkins *et al*. 1984). In both strains, up to 10% of nuclei show two nucleoli (slightly smaller than normal) while the remaining nuclei contain a single larger nucleolus, interpreted as resulting from fusion. The extracted conidial nuclei may also be used for *in situ* hybridization studies.

References

Lu, B. C. 1993. Spreading the synaptonemal complex of *Neurospora crassa*. Chromosoma 102: 464-472.

Perkins, D. D., N. B. Raju, and E. G. Barry. 1980. Chromosome rearrangement in Neurospora that produces viable progeny containing two nucleolus organizers. Chromosoma 76: 255-275.

Perkins, D. D., N. B. Raju, and E. G. Barry. 1984. A chromosome rearrangement in Neurospora that produces segmental aneuploid progeny containing only part of the nucleolus organizer. Chromosoma 89: 8-17.

Raju, N. B. 1998. A quick silver-staining method for conidial nuclei, and its usefulness for studying the behavior of the nucleolus organizer in Neurospora. Neurospora 1998 (Asilomar conference), Abstract 34, p. 25.

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