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NOTES

Rapid Nuclear Staining Method for *Saccharomyces cerevisiae*

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Mithramycin was used to stain nuclei in mitotically dividing and sporulating yeast.

Observation of the shape and position of the yeast nucleus has proved useful in monitoring the mitotic cell cycle and sporulation (15, 20), in classifying temperature-sensitive mutants (7, 9), and in assessing methods for the fractionation of populations into cells of different ages (18). However, conventional nuclear staining methods (Giemsa [16], acetic orcein [6], acridine orange [17]) for yeast involve several hours and multiple manipulations even when modified to study only the position of the nucleus rather than its internal structures (7, 8). Recently, more rapid methods for staining fungal nuclei have been reported, all of which require a critically timed hydrolysis of ribonucleic acid (4, 10, 13). We report here a rapid, one-step procedure for fluorescent staining of the yeast nucleus with mithramycin.

The specific binding and fluorescence properties of mithramycin (5, 19) make it unnecessary to hydrolyze ribonucleic acid or to remove the stain before observation. To stain vegetatively growing cells, a sample of culture is added to an equal volume of 50% (vol/vol) aqueous ethanol containing mithramycin (0.4 mg/ml) and 30 mM $MgCl_2$. After 15 min, counterstain is added, if desired, and a drop is examined as a wet mount. For staining cells during meiosis, a sample is centrifuged and suspended in 70% ethanol. After 20 min, 2 volumes of aqueous 22.5 mM $MgCl_2$ containing mithramycin (0.6 mg/ml) are added. After 20 min, counterstain is added and wet mounts are observed. Acridine orange is used as the counterstain at a final concentration of 1 μg/ml in both cases. Solutions are prepared directly in vials, provided by Pfizer Ltd., that contain "carrier" material in addition to 2.5 mg of mithramycin. It should be noted that in the final staining solutions the concentration of mithramycin is 0.2 mg/ml for vegetative cells and 0.4 mg/ml for meiotic cells. In both cases the final concentration of $MgCl_2$ is 15 mM.

Preparations were examined with a Leitz vertical-illumination fluorescence microscope with blue excitation filter KP400, a 455-nm dichroitic mirror, and suppression filter K460 (position 2). Cells were photographed with Kodak Tri-X film after being immobilized in 0.5% Noble agar (1). The filters were chosen to minimize photoinactivation rather than to maximize brightness. Routine observation does not require photography.

Figure 1 illustrates cells of the haploid strain S288C (ATCC 26108) predominantly in mid-nuclear division, i.e., with the nucleus in the neck between the mother cell and bud. Figures 2 and 3 illustrate sporulating diploids. The results are similar to those described previously with Giemsa staining (15), although the present method does not easily stain nuclei in mature ascospores at the completion of sporulation.

Various modifications of the method were found satisfactory. Counter-staining is unnecessary if simultaneous mercury and tungsten lamp illumination is used with an OG 2 filter in the tungsten light path. Excitation filter KP500, a 510-nm dichroitic mirror, and suppression filter K515 (position 3) may be used with a 35%-transmission neutral-density filter in the mercury light path. Cells may be harvested and stored in 70% ethanol or 25% ethanol at 4 C before being stained or in staining solution either in tubes or as wet mounts sealed with Permount. Sealed preparation stored at 4 C did not lose contrast or brightness for over a month. Cells may be fixed with either absolute ethanol and acetic acid (3:1, vol/vol) (11) or absolute ethanol and acetone (1:1, vol/vol) (4). However, in these instances, fixative must be removed and the cells must be washed with 25% ethanol before staining. Fixation may be at room temperature for 10 min or overnight at 4 C. Nuclei in mature ascospores become brightly stained after about a week in stain at 4 C.

A note of caution must be added. Adding acridine orange before all of the nuclei are stained may retard subsequent staining. Since

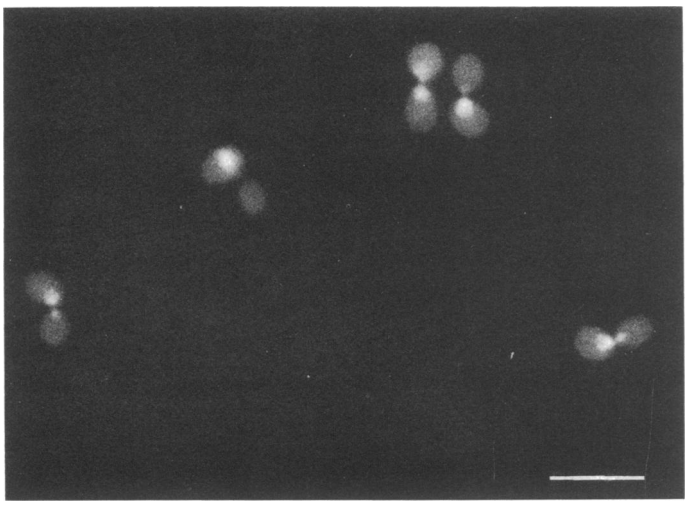

FIG. 1. *Vegetatively dividing S288C. Log-phase cells in minimal medium (2) were exposed to 0.1 M hydroxyurea to accumulate cells in mid-nuclear division (18). A sample was stained, imbedded in agar, and photographed. The marker represents 10 μm.*

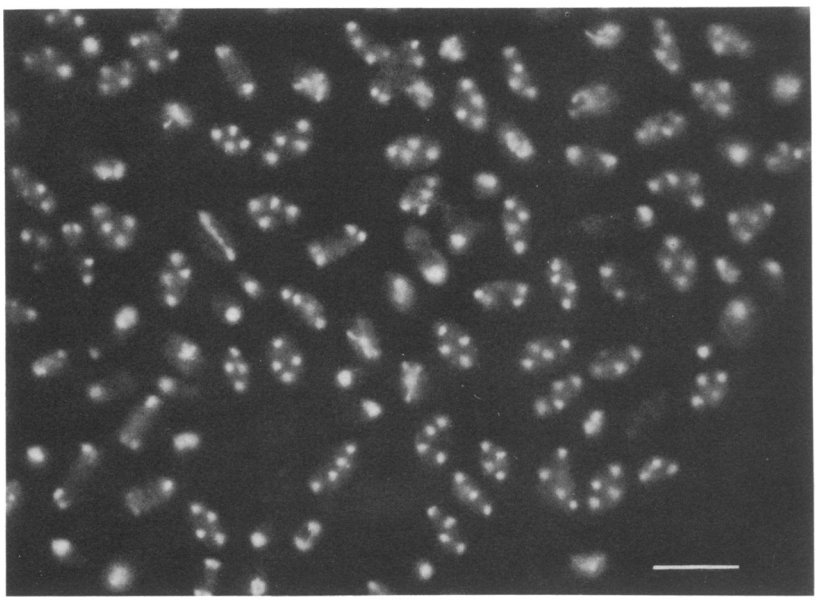

FIG. 2. *Sporulating SK-1. Samples removed 5 and 7 h after inoculation into sporulation medium (10) were suspended in 70% ethanol and refrigerated overnight. The samples were then stained and mixed to exhibit various phases of meiosis. Part of the mixed sample was imbedded in agar and photographed. The marker represents 10 μm.*

the time required to stain all of the nuclei varies with different populations (e.g., log phase, resting, sporulating), a preliminary experiment with a given strain and medium may be advisable to determine the proper time for adding counterstain.

The following procedures were found unsatisfactory: (i) fixing in saline-formalin overnight or in Helly fixative or Carnoy fixative for 10 min; (ii) staining nuclei with quinacrine mustard dihydrochloride (3), 33258 Hoechst (12), or daunomycin (14) for 60 min in the solu-

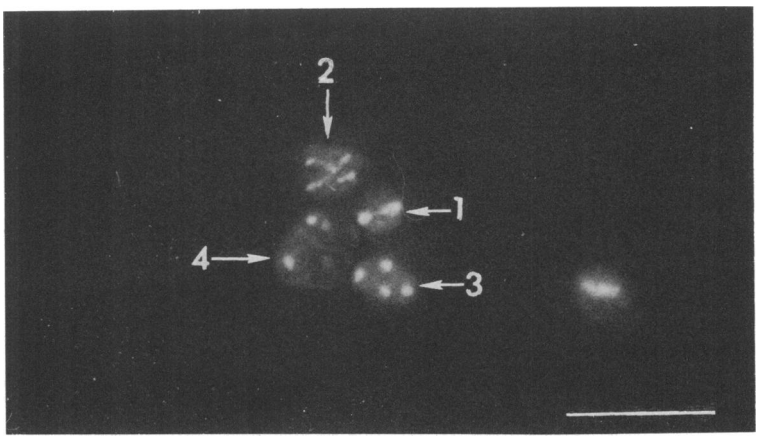

FIG. 3. *Sporulating SK-1. Stained cells from the mixed sample illustrated in Fig. 2 were photographed at higher magnification. Cells 1, 2, and 3 contain one dividing nucleus, two dividing nuclei, and four nuclei, respectively. Cell 4 contains two brightly stained nuclei and two ascospores in which the stain does not penetrate easily. The marker represents 10 μm.*

tions prescribed in the appropriate references after initial fixation with 70% ethanol, formalin-saline, or Carnoy solution in each case.

The method described here is designed primarily for observing the stages of nuclear division rather than for studying the internal structures of the nucleus. It has the advantages of rapidity and simplicity. Thus, mitotic cell cycles or sporulation can be monitored at the time populations progress through these processes rather than after they are completed. Also, large numbers of samples can be stored and processed later to screen mutants or to assess synchrony.

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