NUCLEAR CHANGES IN THE LIFE CYCLE OF SACCHAROMYCES[1]

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Wager (1898) and Wager and Peniston (1910) described the nuclear division prior to the formation of buds and spores in Saccharomyces as an amitotic process. Guilliermond (1903 and 1910) described it as amitotic, but his conception of the nucleus differed from that of Wager and Peniston in that he considered the chromatinic body, which they described as the nucleolus, to be the nucleus. Badian (1937) also considered the chromatinic body to be the nucleus, but he did not agree that its division was amitotic. He considered that the yeast cell contained two chromosomes which divided by longitudinal splitting in mitosis, and in copulation the two chromosomes of each of the gametes fused end to end rather than associating to form a pair according to the usual method. According to his conception the yeast cell contained two chromosomes regardless of its degree of ploidy. McClary *et al.* (1957) have shown that there is a direct relationship between the chromosome number of Saccharomyces and its degree of ploidy, and that the haploid number is probably four. It is evident that Badian mistook the double appearance of an optical section of the chromatin covered spindle in budding cells (Lindegren *et al.*, 1956) or the two spindles of meiosis II (figure 14 of this paper) as individual chromosomes.

Nagel (1946) made a study of budding and sporulation in yeast using Robinow's Giemsa technique and the Feulgen technique. She called the vacuole the magnicarp and the spindle the parvicarp. She stated that vegetative division of the parvicarp did not appear typically mitotic, but that the prophase of meiosis resembled conventional configurations. Winge (1935) and Lindegren (1949) described the life cycle of Saccharomyces.

Preparations by the modified Carnoy-perchloric-Giemsa technique (Lindegren *et al.*, 1956) described herein indicate that the nucleus of Saccharomyces is like that of other Ascomycetes and that conventional chromosomal configurations of its division are demonstrable.

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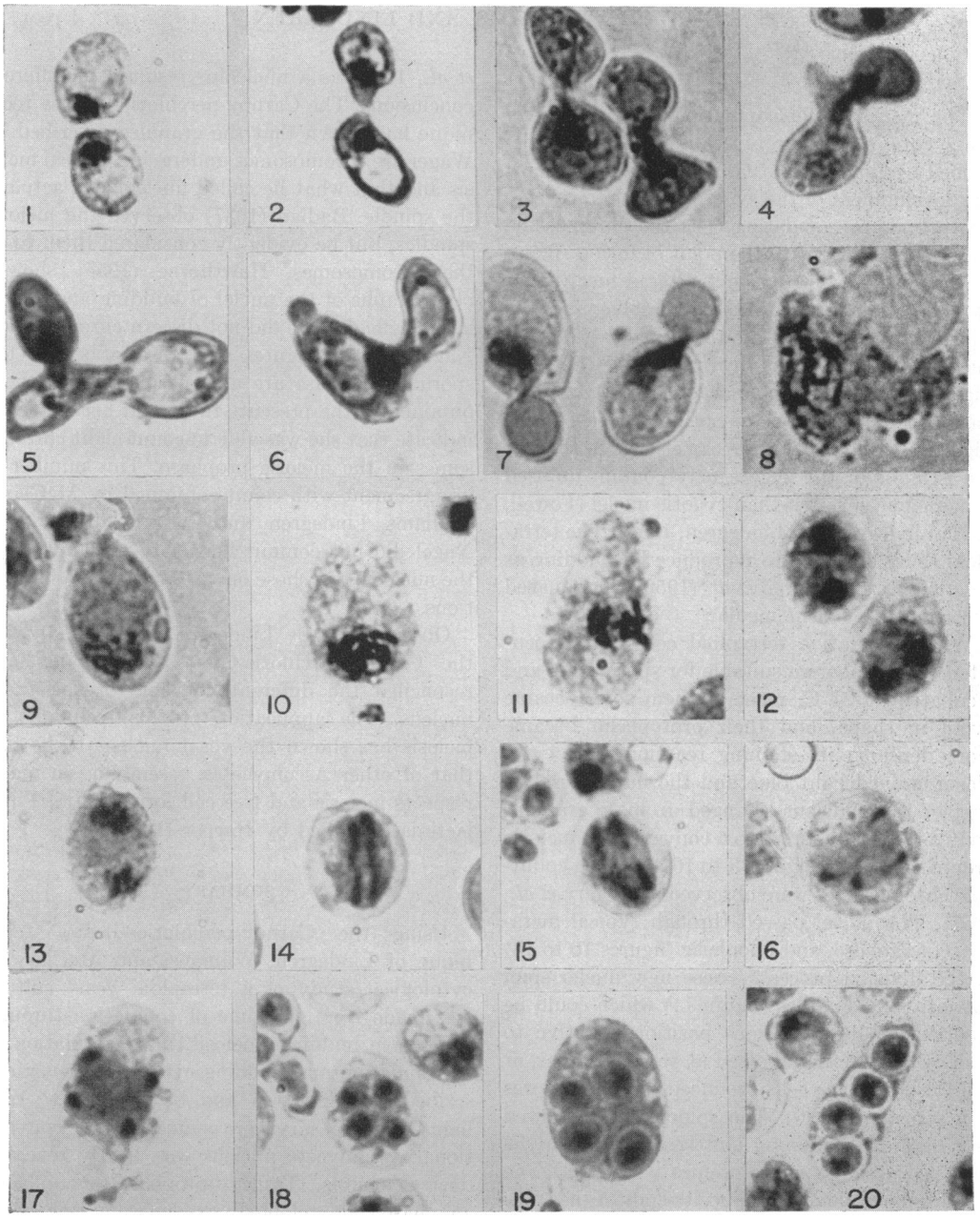
EXPERIMENTAL METHODS

Diploid (homozygous for mating type) cultures 11294 a/a and 11296 α/α (Lindegren and Lindegren, 1951) were grown on agar slants containing glucose, 20 g; basamin (Anheuser-Busch), 5.0 g; $(NH_4)_2SO_4$, 2.0 g; KH_2PO_4, 2.0 g; $MgSO_4$, 0.5 g; agar, 20 g; and distilled water to one liter. After incubation for 24 hr at 30 C, a large loopful of each culture was mixed together on a series of slants of the same medium and incubated at 30 C for varying periods of time to permit copulation and the formation of budding zygotes. Slants were sampled at hourly intervals for 6 hr, fixed in modified Carnoy solution, and stained by the perchloric-Giemsa method of Lindegren *et al.* (1956). Some of the mating mixtures were allowed to incubate for 24 hr to produce mature, tetraploid cells. Agar plates were streaked from these cultures and incubated until tetraploid colonies could be obtained and inoculated onto fresh agar slants.

Synchronous sporulation was obtained by inoculating a series of slants of the modified sporulation medium of Stantial (1928, 1935), Elder (1933, 1937), and Adams (1949) from 24-hr tetraploid slant cultures and incubating from 1 to 24 hr, after which time most of the cells had developed into four-spored asci. Slants were taken at hourly intervals and the cells were fixed and stained in order to follow the cytological changes.

All photographs were made from wet mounts. Permanent mounts of comparable clarity have recently been made as follows: A loopful of the stained preparation is squashed under a cover slip and blotted. The cover slip is removed and placed on a clean slide in a drop of "diaphane" (Will Corporation, New York, N. Y.) mounting fluid, and the excess fluid is removed by blotting with bibulous paper. The slide may also be used by placing a clean cover slip in diaphane over it and blotting. These slides are available upon request.



Figures 1 and 2. The orientation of diploid mating type cells with their nuclei adjacent to each other and the extension of copulation tubes.

Figure 3. Plasmogamy has been completed; karyogamy has been initiated.

Figures 4 and 5. Karyogamy has been completed and the hybrid bud is appearing.

Figure 6. A bud appears from the pole of the zygote instead of the bridge. It may not be a hybrid bud.

Figure 7. Vegetative budding tetraploid cells.

Figures 8, 9, and 10. Smear preparations of crushed cells late prophase to metaphase preceding the formation of spores.

Figures 11 and 12. Late anaphase and telophase of meiosis I.

Figure 13. Prophase of meiosis II.

Figures 14, 15, 16, and 17. Early and late telophase of meiosis II. Discrete chromosomes are visible in figure 15.

Figures 18, 19, 20. The formation of spore walls in four-spored asci. Epiplasm is clearly defined in figure 19.

RESULTS

The mechanics of copulation and budding of the zygote in Saccharomyces is essentially as described by Lindegren (1951). Figures 1 to 5 show the development of the zygote and the first vegetative bud. In copulating cells the chromosome masses are adjacent to the point of fusion (figure 1). The copulation tubes extend and fuse (figure 2). The walls between them dissolve and the nuclei fuse (figure 3). The tetraploid zygote produced tetraploid cells by vegetative budding (figures 4 and 5). In some zygotes there was budding at the poles of one or both of the fused cells (figure 6). Buds which appear elsewhere than at the isthmus of the zygote may contain unfused gametic nuclei rather than zygotic nuclei (Fowell, 1951; Lindegren and Lindegren, 1954). The tetraploid cells continued to reproduce by budding as described by Lindegren *et al.* (1956) when placed on ordinary nutrient media.

When vegetative tetraploid cells were inoculated onto a medium suitable for sporulation and incubated at 24 C, most of them soon became ovoid in shape, and their protoplasm became more dense in its staining reaction. The cytoplasm stained light blue and the nucleus red or purple. The nucleus changed from a granular body as seen in figure 7 to a conventional meiotic prophase nucleus (figures 8 to 10) in which countable chromosomes were observed (McClary *et al.*, 1957). The nuclei passed through typical metaphase, anaphase, and telophase (figures 10 to 13) in the division process. These new nuclei spun out into long spindles (figure 14) which could be observed to lie at various positions relative to each other (parallel, crossed at various angles, or end to end). Some cells produced their ascospores in a row (figure 20). The spindle was observed as a light pink staining matrix along which the deep red staining chromosomes were seen. The chromosomes moved to opposite ends of the spindle, and the residual fibers dissolved leaving four separate nuclei in a dense protoplant (figures 15 to 17). Free cell formation, characteristic of the Ascomycetes, occurred resembling that described by Harper (1905) resulting in the formation of four-spored asci (figures 18 to 20).

DISCUSSION

Wager's figures (1898) show the meiotic phases almost exactly as we have photographed them, but his interpretation of the spindle (Lindegren *et al.*, 1956) as a nucleolus resulted in different conclusions. The Carnoy-perchloric-Giemsa technique has shown that the granules described by Wager are chromosomes undergoing typical meiosis and that what he called nucleolus is actually the spindle. Badian (1937) observed the meiotic spindles, but he evidently considered them to be the chromosomes. Hawthorne (1955) showed photographs of the nuclei of budding and sporulating cells, but he did not discern chromosomes. Nagel's (1946) figures and descriptions of the sporulation phase are almost identical to those obtained in the present study, and her drawings indicate that she was able to count eight chromosomes in the meiotic prophase. This number is in agreement with counts obtained by McClary, Williams, Lindegren, and Ogur in diploid cells. Nagel did not commit herself to a definition of the nucleus, but chose only to record her observations.

Observations by Lindegren *et al.* (1956) with the Carnoy-perchloric-Giemsa technique have reconciled the different concepts of the yeast nucleus. The application of this technique to meiosis has shown the yeast nucleus to be like that of other Ascomycetes, resembling in many respects meiosis and free cell formation of Phyllactinia described by Harper (1905).

SUMMARY

Using the Carnoy-perchloric-Giemsa technique of Lindegren, Williams, and McClary, a cytological study of a tetraploid yeast culture was made from the time of copulation through vegetative budding, meiosis, and sporulation.

Copulation and budding cycles previously described by Lindegren and by Lindegren, Williams, and McClary were confirmed. The conventional Ascomycete nucleus was clearly revealed during meiosis. Typical prophase, metaphase, anaphase, and telophase nuclei were seen. Free cell formation of ascospores described by Harper in the mildews occurs in yeasts.

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