

THE NUCLEUS IN THE LIVING YEAST

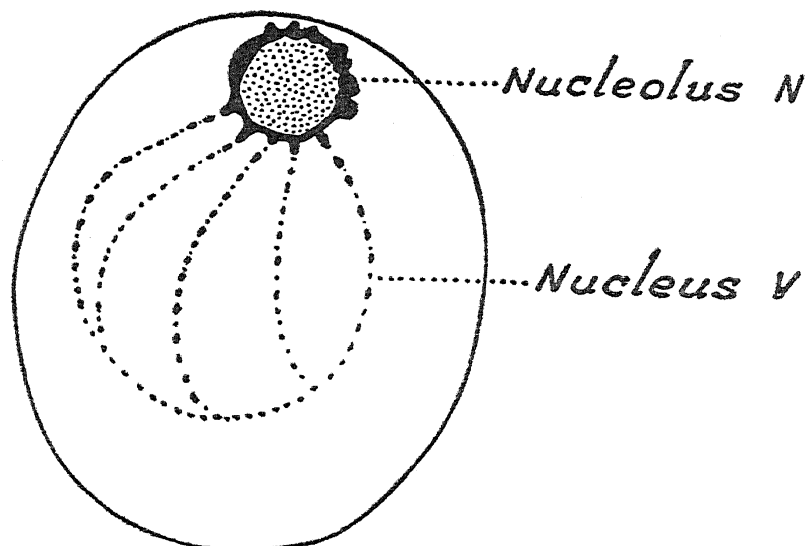
By (MISS) SARASWATHY ROYAN AND M. K. SUBRAMANIAM, F.A.Sc.

(Cytogenetics Laboratory, Department of Biochemistry, Indian Institute of Science, Bangalore-3)

Received February 23, 1956

INTRODUCTION

IN 1910 Wager and Peniston identified the vacuole in the yeast cell as its nucleus and a homogeneous body lying in the cytoplasm but in close contact with the vacuole as the nucleolus (Text-Fig. 1). During cell division the 'nucleolus' is said to constrict into two equal or unequal parts, one of which together with a part of the vacuole passes into the bud.



TEXT-FIG. 1. After Wager and Peniston, 1910.

The identification of these cell organelles has been in dispute and the following table would give an idea of the interpretations of the various investigators.

There appears to be a large measure of agreement regarding the identification of the homogeneous body as the nucleus. The differences relate only to the nuclear nature of the vacuole (1, 2 and 6 in table).

According to Wager and Peniston (1910) the 'nuclear vacuole' disappears during sporulation and the spore nuclei are said to originate by the division of the 'nucleolus' (p. 75). Nagel (1946) could not obtain any evidence that the vacuole takes part in meiotic phenomena. The "parvicorp" on the other hand was observed to exhibit changes suggestive of meiosis,

			Homogeneous Body	Vacuole
1.	Janssens and Leblanc, 1898 Part of nucleus	Part of Nucleus
2.	Wager and Peniston, 1910 Nucleolus	.. Nucleus
3.	Guilliermond, 1920 Nucleus	.. Vacuole
4.	Winge and collaborators, 1954 Nucleus	.. Vacuole
5.	Badian, 1937 Nucleus	.. Vacuole
6.	Lindegren, 1952 Centrosome	.. Nuclear Vacuole
7.	Nagel, 1946 Parvicorp	.. Magnicorp
8.	Subramaniam and collaborators, 1946 Nucleus	.. Vacuole
9.	DeLamater and collaborators, 1950 Nucleus	.. Vacuole
10.	Lietz, 1951 Nucleus	.. Vacuole
11.	Mundkur, 1954 Nucleus	.. V acuole

Lindegren (1945) revived the old concept of Wager and Peniston (1910) that the vacuole is the nucleus of the yeast cell, but in deference to modern ideas regarding the origin and location of the nucleolus, identified the 'nucleolus' of Wager and Peniston as the 'centrosome'. During sporulation he observed an "apparent disappearance of the vacuole" and experienced difficulty in distinguishing the 'chromosomes' from the 'centrochromatin'. Widra and DeLamater (1955) conclude that the vacuolar contents are ergastic in nature.

To reconcile these divergences one has to locate the organelles under dispute in living cells. Wager and Peniston (1910) remark that the 'nucleolus' is generally invisible in living cells but that occasionally "it can be distinguished as a slightly refringent body in contact with the central vacuole" (p. 49). Lindegren and Rafalko (1950) also report that only occasionally could the 'centrosome' be observed as an "opaque area" in the cytoplasm.

MATERIAL AND METHODS

On the belief that a rational approach to a study of the staining reactions and behaviour of these cell organelles would be in a strain where these two structures could be observed together in unstained living cells, an extensive

survey of the yeast cultures in our collection was carried out. The growth medium employed was barley malt wort of S.G. 1.020 and pH 4.6–4.8. Cells from cultures of varying ages were examined first under a Zeiss Phase Contrast Research Microscope and later with a Bausch and Lomb microscope under ordinary as well as dark ground illumination. The photographs were taken with a Leica attachment on Kodak 35 mm. 'Microfile' film. The negatives were enlarged to varying degrees.

OBSERVATIONS

In cells of a 120-hr. culture of a strain of *Saccharomyces cerevisiae*,—obtained through the kindness of Dr. Wickerham,—the two structures described by Wager and Peniston (*N*, *V*, Text-Fig.) could easily be observed. Photos 1 and 2 show their appearance under phase contrast. The vacuole (*V*) appears free of inclusions, but bordering it are grains of different sizes. The corpuscle (*N*) associated with the vacuole (Photos 1 and 2) is generally globular. In Photo 1 it lies partly over the vacuole while in Photo 2 it is merely in contact with it.

Photography with a light microscope is relatively difficult (Photo 3). Granules could be seen arranged irregularly along the boundary of the vacuole. The picture seen under dark ground illumination is superposable on those of phase contrast and light microscopes. According to Wager and Peniston (1910) the vacuole is not delimited from the cytoplasm by a membrane. Examination under dark ground illumination suggests the existence of a membrane surrounding not only the vacuole (*VM*, Photos 5 and 6) but also the body associated with it (*NM*, Photos 4 and 5).

Cell division was rather rare in the 120-hr. culture and the appearance of a budding cell under dark ground illumination is illustrated in Photo 6. The bud is free of any inclusions. The mother cell has a huge vacuole and near the bud is the nucleus (*N*). There appears to be a membrane (*M*) between the vacuole and the nucleus.

DISCUSSION

Wager and Peniston observe: "The large size of the nuclear vacuole (Figs. in Pl. VI) is so disproportionate to the size of the cell and contains such a large quantity of clear sap in proportion to the very diffuse nuclear network, that failure to recognize it as a nucleus is not perhaps astonishing. But the extraordinary activity of the yeast cell at various stages in development explains the unusual size of the nuclear vacuole, and in the light of the large vacuolar nuclei associated with gland or other cells which exhibit very active metabolism, affords additional proof of its nuclear nature" (pp. 55–56)

(*cf.*, Royan and Subramaniam, 1954). This suggestion is further elaborated by them in the concluding part of the paper. Since the vacuole plays no part in sporulation they conclude that the nuclear vacuole is concerned purely with cell metabolism acting as a store-house for the accumulation and elaboration of chromatin. If their interpretation is accepted, then, on analogy with the condition in Ciliates the 'nucleolus' (= 'centrosome' of Lindegren) would correspond to the germinal micronucleus and the vacuole to the vegetative macronucleus.

Cytological analysis has all along been on the assumption that yeast is uni-nucleate (Subramaniam and Royan, 1954). Can it be that yeast is bi-nucleate?

SUMMARY

1. There appears to be a large measure of agreement regarding the identification of the homogeneous body in the yeast cell as the nucleus. The differences relate only to the nuclear nature of the vacuole.

2. In a 120-hr. culture of a strain of *S. cerevisiæ*, the appearance of the nucleus and vacuole under ordinary, phase contrast and dark ground illumination is described. Each appears to possess a membrane delimiting it from the cytoplasm. Can it be that yeast is bi-nucleate?

REFERENCES

1. Badian, J. .. *Acad. Polon. Bull.*, 1937, **1B**, 61-87.
2. DeLamater, E. D. .. *Jour. Bact.*, 1950, **60**, 321-32.
3. Guilliermond, A. .. *The Yeasts*, Trans., F. W. Tanner, 1920.
4. Janssens, F. A. and Leblanc, A. .. *Cellule*, 1898, **14**, 201-43.
5. Lietz, K. .. *Arch. Mikrobiol.*, 1951, **16**, 275-302.
6. Lindegren, C. C. .. *Symp. Soc. Expl. Biol.*, 1952, **6**, 277-89.
7. — and Rafalko, M. M. .. *Expl. Cell. Res.*, 1950, **1**, 169-87.
8. Mundkur, B. D. .. *Jour. Bact.*, 1954, **68**, 514-29.
9. Nagel, L. .. *Ann. Mo. Bot. Gdn.*, 1946, **33**, 249-73.
10. Royan, S. and Subramaniam, M. K. .. *Curr. Sci.*, 1954, **23**, 315-19.
11. Subramaniam, M. K. .. *Proc. Natl. Inst. Sci., India*, 1946, **12**, 143-49.
12. — and Royan, S. .. *Curr. Sci.*, 1954, **23**, 351-54.
13. Wager, H., and Peniston, A. .. *Ann. Bot.*, 1910, **24**, 45-84.

14. Widra, A. and DeLamater, E. D. *Am. J. Bot.*, 1955, 42, 423-35.
15. Winge, O. and Roberts, C. *C.R. Lab. Carlsberg. Ser. Physiol.*, 1954, 25, 285-327.

EXPLANATION OF PLATE

PHOTOS 1 and 2. Living cells under phase contrast.

PHOTO 3. Living cells under ordinary illumination.

PHOTOS 4, 5 and 6. Living cells under dark ground illumination.

N, Nucleus; V, Vacuole; NM, Nuclear Membrane; VM, Vacuolar Membrane.

Range in cell size $4-6 \mu \times 6-8 \mu$.

