THE YEAST NUCLEUS

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Introduction

The discovery of the nucleus in a living yeast cell (Nägeli, 1844) closely followed that of Brown (1831; Sharp, 1934) in plant cells. But yet, while the progress in our knowledge of the structure and behaviour of the nuclei of higher organisms has been orderly and phenomenal, the original identification of the nucleus of the yeast cell itself has been questioned and a variety of cell organelles have been suggested to represent its nuclear 'apparatus'. This is well illustrated by the mutually contradictory conclusions drawn from recent publications in the two latest reviews on the subject (Nickerson, 1957; Winge and Roberts, 1958).

While the nucleus is visible in many types of living cells of higher organisms, it is generally invisible in living yeast cells. The confused state of our knowledge of its structure is only a corollary to the lack of availability of the living nucleus for comparison and reference. Henneguy's (1896) casual record of having seen a nucleus with a nuclear membrane and a nucleolus, in living and stained preparations of a red yeast, lacked confirmation, since later investigators observed it only as a homogeneous body in living cells. Whether the physiological age of the cells at the time of examination had any relation to this divergence of opinion remained long unexplored.

Disagreements Regarding Identification

When the organelle appears homogeneous in the living condition (Wager, 1898; Wager and Peniston, 1910; Sinoto and Yuasa, 1941; Lindegren and Rafalko, 1950; Mundkur, 1954) and is seen after staining as a dense structure (Wager, 1898; Wager and Peniston, 1910; Mundkur, 1954) or as a vesicle delimited by a membrane and containing formed structures inside (Sinoto and Yuasa, 1941) judgment as to which one of them is the true picture of the nucleus becomes questionable. The nuclear membrane and structures inside are likely to be dismissed as artefacts (Mundkur, 1954) if structural differentiation was not visible in the living condition.

If the organelle appears homogeneous in the living as well as stained preparations, its nuclear nature becomes suspect and is liable to be identified as a 'nucleolus' (Wager and Peniston, 1910) or as a 'centrosome' (Lindegren, 1949). If there are areas staining differentially in that structure they are likely to be considered as lying on the surface of that organelle (Wager and Peniston, 1910; Lindegren, Williams and McClary, 1956).

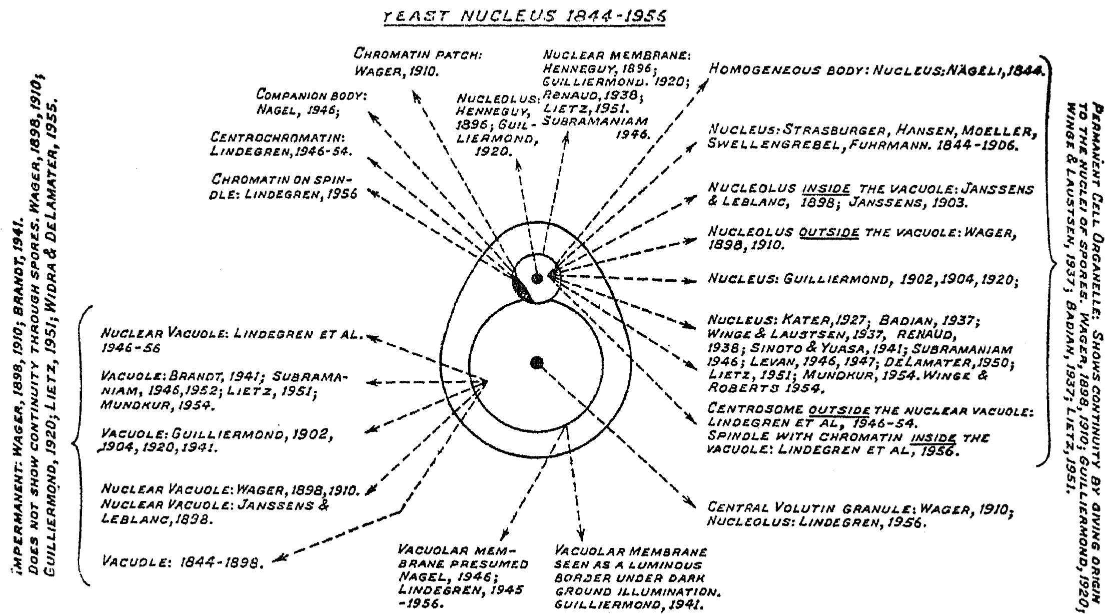
It is in such a context that the vacuole, visible in living cells, began to loom large in discussions on the yeast nucleus. The records of the presence of a network (Wager and Peniston, 1910) or of bodies extending into it (Lindegren, Williams and McClary, 1956), in stained preparations, are at variance with the affinity of the living vacuole for neutral red (Guilliermond, 1941) like the vacuoles of plant cells. The vacuole itself does not appear to be a permanent organelle of the yeast cell (Guilliermond, 1941; Caspersson and Brandt, 1941; Subramaniam, 1946; Aswathanarayana, 1956 *b*, 1958; Thyagarajan, 1956).

The Limitations of Fixation and Staining Procedures

The problem becomes more difficult when, as in many yeasts, the nucleus is invisible in the living condition at the time the investigations are carried out. The identification then of a particular organelle as the nucleus has necessarily to be based on studies of stained preparations under an ordinary microscope or of ultra-thin sections under an electron microscope (Agar and Douglas, 1957; Hashimoto, Conti and Naylor, 1958). The first step in such procedures is the proper fixation of the cells. When there is a doubt as to which organelle is the nucleus, the choice of a fixative becomes arbitrary. When the living nucleus is not available as a 'standard', an evaluation of its reaction to fixatives with a view to choose those which would give a life-like preservation becomes impossible.

This difficulty becomes magnified when it comes to the choice of suitable staining procedures. Hæmatoxylin, the stain of choice of earlier investigators, is a regressive stain. The stage to which the cells have to be destained to reveal the correct picture of the nucleus becomes purely arbitrary and empirical in the absence of the living nucleus for comparison and reference. "The Feulgen technique can lay claim to marked specificity in dye binding but has an analogous weak point in its initial acid hydrolysis step which is likewise of arbitrary duration and intensity" (Nickerson, 1957, p. 34). The Feulgen technique reveals only the location of the DNA in the cell. To determine whether the DNA is uniformly dispersed in the nuclear matrix (Mundkur, 1954) or whether it occupies only a limited area (Lietz, 1951) the living nucleus should be available for comparison. When there is no

agreement as to which organelle of the yeast cell is its nucleus or what its real structure is, it would be futile to discuss supernumerary mitoses and binucleate stages (Winge and Roberts, 1954 *a*, *b*; Roberts and van der Walt, 1959). In the context of the limitations of classical staining procedures, the views of various investigators from 1844–1956 given in *Pictographic Summary I* have only a historical interest.



Pictographic Summary 1

PICTOGRAPHIC SUMMARY 1.

Reconciliation of the Differing Views

In such a dilemma the obvious procedure is to carry out an extensive search of several species and strains of yeasts to see whether a nucleus having a conventional structure could be observed in living vegetative cells under some physiological conditions. The choice of vegetative cells is suggested by the facility with which they could be handled. A search under varying physiological conditions is necessitated by the doubt whethcr the high glycogen and RNA content at some stages of the growth cycle may not be responsible for the invisibility of the nucleus. The resting nucleus was the objective because an accurate knowledge of its structure is a prerequisite for attempts at elucidation of its behaviour during budding and spore formation.

Recent Advances

An extensive search carried out in this laboratory enabled the demonstration side by side of the vacuole and the nucleus in living vegetative cells of three

strains of *Saccharomyces cerevisiæ* (Royan and Subramaniam, 1956; Royan, 1956 *a*, *b*, *c*, 1958 *a*, *b*, *c*; Aswathanarayana and Subramaniam, 1958; Subramaniam, Royan, Thyagarajan, Aswathanarayana and Subramanyam, 1959), one of *Saccharomyces bayanus* (Aswathanarayana, unpublished) and in the living vegetative cells and zygotes of *Saccharomyces carlsbergensis* (Thyagarajan and Subramaniam, 1957 *a*, *b*; Thyagarajan, 1959). To observe the nucleus, the cells have to be aged in the malt wort in which they had grown. The nucleus becomes visible only when the glycogen disappears from the cells and the cytoplasm loses its intense basophilia (Royan, 1958 *b*). During the process of aging, the vacuolar membrane becomes well defined (Aswathanarayana, 1956 *a*, *b*) and the nucleus appears vesicular with a nuclear membrane enclosing formed structures (Royan, 1956 *a*). Often, the intra-nuclear structures appear plastered on to the nuclear membrane, which in consequence presents an irregularly thickened appearance.

Evidence that the nucleus is an extra-vacuolar structure is available from living cells. Under dark ground illumination the nuclear and vacuolar membranes delimit them from each other (Royan, 1956 *b*, 1958 *b*). When the cells are stimulated with fresh media the vacuoles disappear from view (Aswathanarayana, 1958). During the earlier stages of stimulation the encroaching cytoplasm separated the nucleus from the vacuole (Royan, 1956 *c*). Differential staining of the vacuolar membrane is difficult. But rare instances were available where the vacuolar membrane separated from the cytoplasm during the processing of the hæmatoxylin stained smears to make them permanent (Royan, 1958 *b*). In such cells the nucleus remained in the cytoplasm while the contracted but intact vacuolar membrane was seen lying free inside the vacuolar area (Subramaniam *et al.*, 1959).

When cells with visible nuclei are stained vitally with a proper dilution of neutral red, the vacuole alone has an affinity for the dye indicating that the nucleus and the vacuole are unrelated structures (Thyagarajan, 1958 *a*, *b*). During the segregation of neutral red by the vacuole, the vacuolar membrane was occasionally coloured by the dye.

The identification of the intra-nuclear structures into distinct categories necessitates fixation and staining (Photo 1). Since the vacuole has loomed large in discussions on the yeast nucleus, the fixative chosen should give a life-like preservation of the cell organelles. Most of the fixatives distort or destroy the vacuole. When preservation of one organelle is not exact, there would be legitimate doubts whether the same may not be the case with the others also. The situation becomes complicated when the nucleus is not visible in the living cells and the fixative distorts the prominent vacuole

(Ganesan and Swaminathan, 1958). An evaluation of the action of fixatives using cells with visible nuclei as the standard was therefore rendered imperative.

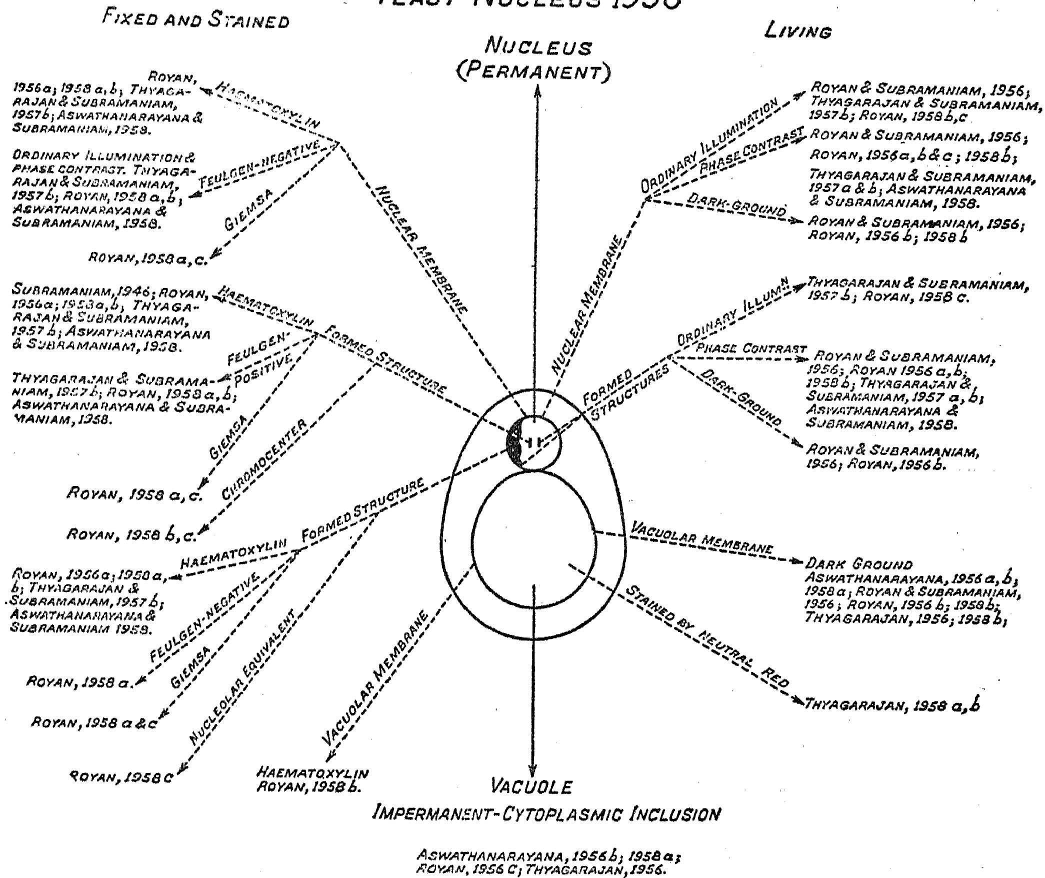
Iodine formaldehyde acetic solution when used in proper dilution gives a life-like preservation (Royan, 1956 *a*, 1958 *a*; Thyagarajan and Subramaniam, 1957 *b*; Aswathanarayana and Subramaniam, 1958). Correct fixation is but the first step in the analysis. Heidenhain's hæmatoxylin reveals all the structures visible in the living nuclei. The vacuole was bereft of stainable structures. When the cells are stained by the Feulgen technique, the stained region was seen to occupy only a portion of the nucleus. To determine the orientation of the stained region with reference to the nuclear membrane, examination under phase contrast was often necessary.

The identification of the Feulgen positive area as the whole nucleus on the assumption that the DNA is uniformly dispersed in the nuclear matrix, and the corollary that the small size of the nucleus (*ca* $0 \cdot 5\,\mu$) precludes any possibility of studying the yeast chromosomes (Winge and Roberts, 1954 *a*, *b*) find little support in the relatively large size of the nucleus (*ca* $2 \cdot 0\,\mu$) in living cells and the absence of any uniform staining of its matrix by the Feulgen stain. In fact, intra-nuclear structures could be clearly distinguished from bi-nucleate stages (Royan, 1958 *b*). The vacuole was Feulgen negative and hence is only a cytoplasmic inclusion.

A comparison of hæmatoxylin and Feulgen preparations showed that the nuclei contain Feulgen positive as well as Feulgen negative structures. The Feulgen positive areas are the chromocenters while the Feulgen negative structures are the equivalents of the nucleoli of higher organisms. The Giemsa stain has recently come into vogue in the study of the yeast nucleus. The general procedure is to stain the fixed smears after hydrolysis. The picture obtained is intermediate between hæmatoxylin and Feulgen preparations (Royan, 1958 *b*). But if unhydrolysed smears are stained with Giemsa, the chromocenters could be distinguished from the nucleolar equivalents by the differences in their colour (Royan, 1958 *c*; Subramaniam *et al.*, 1959).

The recent advances in our knowledge of the yeast nucleus based on a strict comparison of living with stained preparations would emphasize that the nucleus of yeast has a conventional vesicular structure (Subramaniam *et al.*, 1959) with a nuclear membrane, chromocenters and nucleolar equivalents (*Pictographic Summary II*, 1956).

PICTOGRAPHIC SUMMARY 2.



Pictographic Summary 2

SUMMARY

The disagreements on the question whether the nucleus of yeast is an organelle unrelated to the vacuole or whether it is the vacoule itself (*Pictographic Summary I*, 1844–1956) is only a corollary to the limitations of fixation and staining procedures carried out on cells in which the former was invisible. A reconciliation is possible if investigations are confined to cells in which both the organelles are visible. The extra-vacuolar nucleus has a conventional vesicular structure with a nuclear membrane, chromocenters and nucleolar equivalents. The vacuole, which has a limiting membrane in older cells, is Feulgen negative and segregates neutral red in living cells. It is therefore a cytoplasmic inclusion. A strict comparison of living with stained preparations is presented in *Pictographic Summary II*, 1956.

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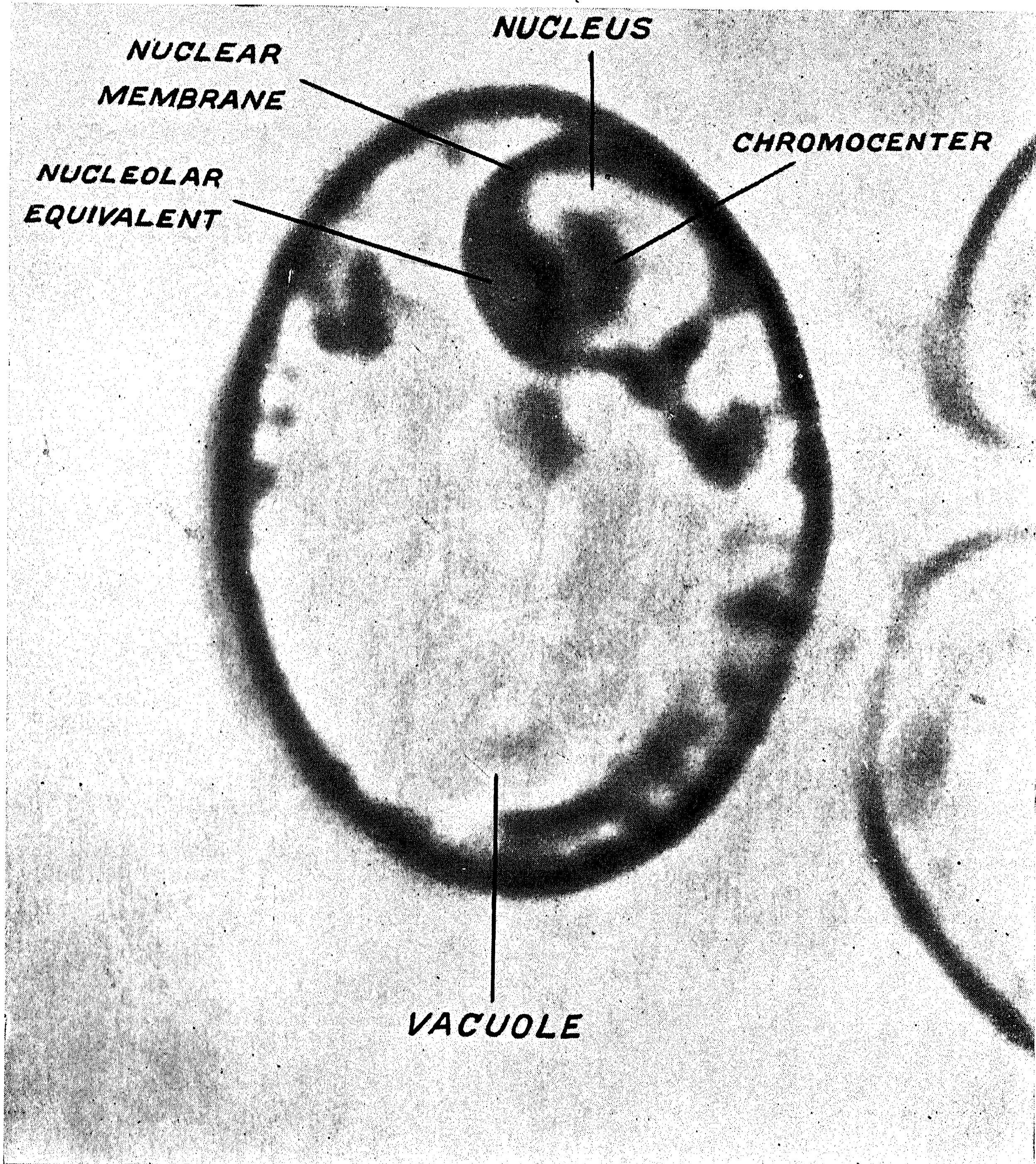

A Living Yeast Cell.
Ordinary Illumination. × 12,000

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