were plated, for 100 plaques on non-irradiated bacteria, 600 plaques appeared on irradiated bacteria.

The control plaques showed that the stock of T 3 used contained two *h* mutants among 12000 h^+ particles. These *h* mutants give, on mixed B + B/3*a* indicator, plaques that are clear right from the beginning of their growth. Among the phages reactivated by UV'd bacteria, there were 61 *h* mutants among 30000 h^+ particles, a proportion twelve times larger than that of the control.

The plaques of *h* mutants observed on irradiated bacteria were not sectored. Some of these plaques were picked, when very young, and analyzed. Each plaque was found to be pure within the accuracy of our analysis (1%) but different plaques were due to *h* mutants differing their plaque morphology.

In addition to these *h* mutations, others were observed showing diverse plaque morphologies, the increase over the control being again of the order of 10.

We conclude that mutations in virulent bacteriophage can be induced by ultraviolet irradiation of the phages and of the bacteria in which they shall multiply. Thus the production of mutations by ultraviolet irradiation is not restricted to temperate, inducible bacteriophage.

We wish to express our thanks to Miss L. HUBER for her assistance during these investigations.

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Résumé

Il est possible d'induire des mutations chez le bactériophage virulent T3. Il faut pour cela irradier avec des rayons ultraviolets *et* les bactériophages *et* les bactéries dans lesquelles ils se multiplieront. Les mutations dont on a mesuré la fréquence concernaient le spectre d'action des phages (T3h), mais on en a observé d'autres agissant sur la morphologie des plaques.

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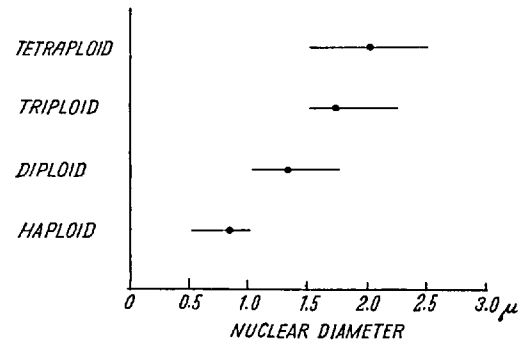
Interphase Nuclei and Cell Sizes in a Polyploid Series of Saccharomyces

Variations in cell dimensions and frequently in nuclear areas correlated with degrees of ploidy are well known cytological phenomena; they have been reported often in plants though their occurrence in higher animals is less common[1]. Similar information concerning the fungal interphase nucleus is lacking, owing mainly to the recentness of intensive genetical interest in the fungi. Undoubted polyploid yeast stocks recovered through genetical procedures have been reported, however, by ROMAN *et al.*[2] and by LINDEGREN and LINDEGREN[3]. These stocks were not investigated cytologically. On the other hand, even cytological claims[4] (unsupported by any genetical analyses) of the existence of an extensive haploid to hexaploid series of yeast provide no information regarding size differences, if any, among the interphase nuclei of these cells. Indeed, had such measurements been made the true dimensions of the nuclei would not have been accurately assessable, since chemical fixatives, such as those hitherto used in yeast cytology, yield appreciably shrunken preparations.

The clones of a polyploid series (kindly supplied by Dr. C. C. LINDEGREN) we now consider, are of single cell origins. Their haploid, diploid, triploid, and tetraploid constitutions have been adequately established by the LINDEGRENS with the use of 6 to 11 genetical markers. The present report concerns cytological preparations of these clones obtained with the ALTMANN-GERSH[1] technique of freezing and drying, of which the superiority over conventional chemical fixatives is universally acknowledged. Advantages of the method consist principally in that shrinkage is absent or is negligible; diffusion currents within the cell are eliminated; and morphological and cytochemical preservation of the fixed cell approximates the living state as closely as is possible with known fixing agents.



Cells for freezing-drying were harvested from fermenting cultures as well as from vigorously aerated liquid medium and frozen in isopentane cooled to – 155° C in liquid nitrogen. Dehydration in vacuum at – 30°C was followed by immersion of the cells in chilled absolute alcohol. Celloidin sections of the fixed material were cut such that their thicknesses only slightly exceeded the longer dimensions of cells of each degree of ploidy, thus ensuring the inclusion of whole as well as sectioned cells. The conventional FEULGEN methods ($HClO_4$ or HCl hydrolysis) as well as the RAFALKO[2] modification were used with or without precipitation of the DNA as the lanthanum salt. Adequately differentiated hematoxylin-pyronin preparations were also obtained. These preparations are characterized by excellent uniformity of staining with the nucleus being very clearly differentiated from the cytoplasm.

The interphase nucleus is defined here as that single, spherical or near-spherical, extra-vacuolar area showing a positive FEULGEN reaction. I have been unable to corroborate LINDEGREN's "nuclear vacuole" view[3], the polymorphic structures identified by him as chromosomes exhibiting, in my experience, neither a FEULGEN positive reaction nor increases in numbers consistent with ploidy, nor even an invariable presence in dividing cells stained with toluidine blue in fixed, or in fresh wet

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	Haploid	Diploid	Triploid	Tetraploid
Nuclear diameter	0·84 μ	1·35 μ	1·73 μ	2·01 μ
Standard deviation	0·0195	0·0663	0·055	0·0833
Mean volume	0·31 c μ	1·288 c μ	2·711 c μ	4·252 c μ

preparations. Figure 1 shows graphically the increments in mean nuclear diameters (indicated by dots) and their ranges of variation in preparations of pure cultures representing each degree of ploidy. The interphase nucleus is seen as a homogeneously stained, vesicular body with no detectable chromatin threads. One hundred such nuclei of each degree in the series were measured in whole, randomly selected cells. Where a nucleus was so closely pressed onto the vacuole as to have a slight dent, its broadest diameter was estimated.

Increments in mean nuclear diameters and volumes are tabulated above.

Since about 22 to 28% of the nuclei in each class were slightly dented by the vacuole, the calculated mean volumes are only approximate. The increments in ranges of variation of diameter, probably associated with growth of the nucleus during interphase[1], will be noted.

These observations are in obvious refutation of LINDEGREN's identifying as the centrosome, a structure here shown to conform to the theoretically expected nuclear area–ploidy relationship. It should be stressed that RANGANATHAN and SUBRAMANIAM's[2] view that even single cell or single spore isolations cannot yield "pure" cultures owing to "somatic doubling" or irregular segregations of split chromosomes, finds no support in the present work. Such mechanisms, if prevalent, would be expected to yield genetical heterogeneity, involving the occurrence of variously sized nuclei, in a culture of initially single-haploid-cell derivation. Meticulous search in haploid preparations has failed, however, to reveal nuclei larger in diameter than haploid nuclei. Neither were cytological differences detected between preparations of aerobically grown and fermenting cultures of haploid origin, the nuclei in both cases falling within the range indicated in Figure 1. This is contrary to SUBRAMANIAMS's thesis that fermenting cells differ cytologically from aerobically active cells and that endopolyploidy is a consequence of fermentative growth.

The size differences between the clones presently studied are as characteristic of the series as are the increases in total nuclear volumes; haploid cells are roundish and measure 2·81 μ in diameter while diploids measure 5·338 × 2·658 μ, triploids 7·041 × 3·591 μ, and tetraploids 7·964 × 4·23 μ. These figures represent the mean of one hundred estimations for each class. Only single, non-budding cells were measured. These observations contrast markedly with those of DURAISWAMI and SUBRAMANIAM[3] (whose "haploids", measuring 10·6 × 7·2 μ, are actually larger than their "tetraploids" measuring 6·9 × 6·6 μ) and indicate that their objection to using cell size as an aid to distinguishing genetical types is not universally valid. It is superfluous to add, in this connection, that yeast geneticists have subordinated morphological criteria to the precise genetical diagnoses possible with biochemical markers but can invariably associate undoubted haploid segregants, in Mendelian as well as non-Mendelian (other than polyploid) asci, with small roundish cells. Matings between compatible segregants reinitiate ascus-size vegetative cells.

Part of this work was carried out at the University of Chicago. I am indebted to Prof. I. GERSH for facilities and advice. A discussion of the nuclear cytology of yeast will appear elsewhere.

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Zusammenfassung

Durch Gefriertrocknung nach ALTMANN-GERSH wurden zytologische Präparate von einer genetisch geprüften polyploiden Serie von Hefe hergestellt. Die gemessenen Werte für Kern- und Zellgrössen stimmen in der theoretisch erwarteten Weise mit dem Grade der Polyploidie überein. Damit ist eine von höheren Organismen bekannte zytologische Erscheinung zum erstenmal für Mikroorganismen demonstriert.

Die Befunde stehen im Widerspruch zu LINDEGRENS Annahme einer «Kernvakuole» und zu SUBRAMANIAMS genetisch nicht fundierten Einwänden gegen die Brauchbarkeit von Einzelsporen-Kulturen, ebenso zur Ansicht des letztgenannten Autors, dass zytologische Unterschiede bestehen zwischen Zellen aus Gärungs- und solchen aus aeroben Kulturen.

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Induction of Resistance to X-Ray Inactivation in Saccharomyces by Pre-Exposure to 2537Å Ultraviolet Radiation[1]

An exploratory study of the effects of combined ultraviolet and X-irradiation revealed a marked increase in the resistance to X-ray inactivation of a portion of the survivors of an ultraviolet irradiated population. In contrast, no change in sensitivity to ultraviolet radiation was observed in the survivors of an X-irradiated population.

A haploid stock, #13894, and a non-sporulating diploid stock, #11296 of *Saccharomyces cerevisiae* from the Carbondale pedigree were employed in this investigation. The ploidy for each stock has been established through genetical analyses and nucleic acid determination[2]. 24 h old clones of each organism were maintained

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