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LIFE SPAN OF INDIVIDUAL YEAST CELLS

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University of California, Berkeley, California

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ABSTRACT

A group of 36 single diploid yeast cells was followed microscopically to observe the maximum number of buds that each could produce. A distribution between 9 and 43 buds was observed, with a median of 24 buds. From the nature of this distribution and the relative sizes of bud scars and cell surface, it is proposed that life-span limitation is a consequence of accumulation of bud scars until the useful surface area was insufficient to maintain normal metabolic processes.

## LIFE SPAN OF INDIVIDUAL YEAST CELLS\*

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University of California, Berkeley, California

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### INTRODUCTION

The division of a yeast cell by budding gives rise to two cells which are identifiable: i. e., the mother cell from which the bud arose, and the daughter cell which developed from the bud. The wall of the daughter cell presumably is synthesized de novo while that of the mother cell retains all or at least part of its identity through the division— an example of linear inheritance.<sup>1</sup> That the wall of the mother cell is not reformed is evidenced by the accumulation of scars associated with each budding event.<sup>2</sup> Bartholomew and Mittwer,<sup>3</sup> Bradley,<sup>4</sup> and Agar and Douglas<sup>5</sup> have studied the budding process in detail by electron micrography. Their photographs indicate a scar area of  $\sim 1\mu^2$ . The average surface area of the cells was 50 to  $100\mu^2$ . If, as Barton indicates, a bud is never formed at the site of a previous scar, then it is quite possible that the life span of individual cells is limited. He observed one cell to bud 23 times but did not observe a cessation of budding. Other factors also might limit the number of generations that a single yeast cell can complete, and it was of interest to look into this further. This report is an account of a study in which a number of single diploid yeast cells was followed until they ceased producing buds, in order to determine an estimate of life span in this organism.

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\*This study is based on work performed under contracts with the U. S. Atomic Energy Commission.

## MATERIAL AND METHODS

A hybrid diploid culture of Saccharomyces cerevisiae, X30, was used for the entire study. The procedure of life-span analysis was as follows:

A layer of growth medium (1/2% yeast extract, 1% dextrose, 2% agar) was spread on the surface of a 22 X 22 mm coverslip and a loopful of a dilute suspension of cells was streaked along one edge of the surface of this agar layer. The coverslip was then inverted over a micromanipulation chamber, maintained at 30°C.

With the aid of a micromanipulator, a number of budding cells were drawn into the central region of the agar. The coverslip was then removed and the agar containing the original inoculum was cut off, leaving only the selected budding cells. The coverslip was again placed in position and the orientation of the buds on each of the mother cells was recorded. The cells developing from these buds were used as starting mother cells and the original mother cells were discarded. As new buds appeared on the selected mother cells, their positions were recorded, and when they had budded again they were removed. Only when the mother and its daughter have each budded can they be separated,<sup>6</sup> so bud removal was always delayed one generation. In order to make an estimate of generation times, the starting mother cells were examined at frequent intervals, and the times of appearance of buds were recorded. Each of the starting mother cells was followed until it failed to bud further. The total number of buds produced was considered the life span.



## RESULTS AND DISCUSSION

A total of 36 mother cells was observed, and the number of buds each produced is shown in the histogram of Fig. 1. A median life span of 24 generations applies to this distribution.

After three or four generations the mother cells were visibly distinguishable from their mature buds. They were considerably larger than first-generation mother cells<sup>3</sup> and surface irregularities were easily seen. These irregularities most likely were due to accumulation of bud scars. Throughout most of the life span of the cells, the generation time varied between 60 and 100 minutes. However, the final one to three divisions of nearly all the cells were found to be much longer in duration, some lasting up to 6 hours. With cessation of budding, most of the cells became granular and some lysed. Both of these observations indicate cell death.

That the surface area of the cells increases with repeated budding suggests the possibility that the bud scar does not replace a portion of the original cell wall but instead fills a region that has been opened up in the cell wall. If this is the case, limitation of life span by depletion of available regions for bud scars would seem less attractive as a hypothesis for a cause of life-span limitation. Also, calculations based on a diploid-cell area of 50 to 100  $\mu^2$  and a bud-scar area of  $\sim 1 \mu^2$  would indicate a longer life span than was observed. A more probable explanation would be that the "useful" surface-to-volume ratio decreases below a level essential to maintain normal metabolic processes, thus precipitating cell death.

Alternative hypotheses based on random occurrence and accumulation of deleterious nuclear events such as nondisjunction or recessive lethal genetic changes, or on random depletion of essential autonomous cytoplasmic constituents, are not plausible since the original population would already be in equilibrium with such events, and the death rate per generation would be expected to remain constant.

It would be of considerable interest to extend these studies by the use of different ploidy cells and also changes in environmental factors such as temperature, medium, and radiation.

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