The Effects of Various Growth Temperatures on Nuclear Division, DNA and RNA in the Budding Yeast, *Kluyveromyces fragilis*

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INTRODUCTION

Most yeasts are able to multiply at or close to 0 °C and have a maximum growth temperature within the range 30 to 40 °C. Phaff, Miller & Mrak (1966) observed that *Kluyveromyces fragilis* (formerly *Saccharomyces fragilis*) does not fit into this general picture, having a minimum growth temperature of 5 °C and a maximum one of 45 °C. The present paper describes the effect of growth at 15, 30 and 40 °C on batch cultures of *K. fragilis*.

METHODS

Organism and cultural conditions. *Kluyveromyces fragilis* (NCYC100) was grown in 2% Oxoid malt extract broth at 15, 30 and 40 °C, with shaking at 160 rev./min. Cultures were grown to the exponential growth phase at each temperature. Under these conditions, the yeasts had a doubling time of 160 min at 15 °C, 90 min at 30 °C and 120 min at 40 °C. Cell number determinations were carried out using a Thoma haemocytometer. (Duffus & Penman, 1973).

Cytochemical and biochemical methods. The time of nuclear division was determined in cells grown at each temperature following Giemsa staining, as described by Duffus & Penman (1973). DNA was estimated in cultures grown at each temperature following the method of Bostock (1970). RNA was estimated using the orcinol method (Mejbaum, 1939).

RESULTS

At 15 °C, 14.7% of cells contained two nuclei. At 30 °C this figure was 32% and at 40 °C it was 20.14%. Therefore at 15 °C, nuclear division occurs at a point 0.80 of the cycle from cell division to division; at 30 °C this point occurs at 0.60 of a cycle, and at 40 °C at 0.74 of a cycle after cell division. The times between nuclear division and cell division are therefore 32 min at 15 °C, 36 min at 30 °C and 32 min at 40 °C.

Table 1 shows the generation time for each growth temperature, the DNA and RNA contents per cell, and the ratio of RNA to DNA. The relationship between the RNA to DNA ratio and the generation time was remarkably constant. At 15 °C it was 1.05, at 30 °C 1.20, and at 40 °C it was 1.15. There is less DNA per cell at 15 °C than at the higher growth temperatures.

DISCUSSION

It should be noted that the above calculation of the time of nuclear division depends on the assumption that the growth rates of individual cells increase exponentially over the cell cycle (see Mitchison, 1971). If this is true, we have a system where the time between nuclear division and cell division is constant and independent of temperature. Any straightforward

Table 1. *Generation times, and DNA and RNA contents of exponentially-growing cells of K. fragilis in asynchronous cultures at different growth temperatures*

	Growth temperature		
	15 °C	30 °C	40 °C
Generation time (min)	160	90	120
$10^{14} \times \text{DNA/cell (g)}$	4.40	6.15	6.40
$10^{13} \times \text{RNA/cell (g)}$	7.40	6.57	8.80
RNA:DNA	168:1	108:1	138:1
RNA/DNA:generation time	1.05	1.30	1.15

chemical system involved in linking nuclear and cell division should show a marked temperature dependence. Consequently one must postulate a more complex system. At its very simplest, such a system would have to involve two reactions, one, initiated by nuclear division, controlling the synthesis of a product necessary for cell division, the other controlling its breakdown. Both of these reactions would have to have equal thermal coefficients.

The apparent constancy of the relationship between the RNA and DNA ratio and generation time is puzzling, and at present no explanation is available to account for this observation. In our results, it reflected a change in the total RNA per cell and, in the 15 °C culture, a decrease in the total amount of DNA per cell. This is surprising because we have previously established (Duffus & Penman, 1973) that the S period of *K. fragilis* is nearly coincident with the time of cell division at 30 °C. Hence, any lowering of the total amount of DNA per cell cannot be explained simply on the basis of the G₂ period (the period between the DNA synthetic period, S, and the period of cell division) being reduced. The 30 °C figure must approximate to the 2c amount of DNA at that temperature and therefore the 15 °C figure represents a marked reduction in the 2c amount of DNA. A possible explanation of the phenomenon is that, at the higher temperatures, one or more of the chromosomes may be replicated more than once during the S period. If this is so, we have a situation analogous to that in *Escherichia coli* where short generation times are characterized by more than one replicating chromosome being present per cell, i.e. generation time is related to the frequency with which replication forks are initiated. Possibly other simple eukaryotes will show the same property. If so, it would be interesting to observe whether there is any correlation with the near absence of histones, as in yeast where only the f_{2a2} fraction appears to be present (Franco, Johns & Navlet, 1974; Penman and Duffus, unpublished).

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