Mutual Inhibition of DNA Synthesis in *a*- and α -cells of *Saccharomyces cerevisiae* during Conjugation

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SUMMARY

Isolation of α mating-type yeasts from a conjugation mixture permitted the direct demonstration of inhibition of DNA synthesis in these cells as a necessary prelude for zygote formation in yeast.

INTRODUCTION

Duntze, MacKay & Manney (1970) and Bücking-Throm, Duntze, Hartwell & Manney (1973) have demonstrated that α -cells produce constitutively an α -factor which inhibits the initiation of DNA replication in *a*-cells and thus synchronizes them. As yet there is no evidence that *a*-cells form an *a*-factor able to inhibit DNA replication in α -cells. Hartwell (1973) has shown that only single, unbudding yeast cells of *a*- and α -type are able to fuse in the conjugation process.

Following our studies on the physiology of conjugation in yeast (Biliński, Litwińska, Żuk & Gajewski, 1973) which enabled us to establish optimal conditions for conjugation and synchronous zygote formation, DNA synthesis has been studied in α -cells separated from such a conjugation mixture.

METHODS

Strains. The following strains were used: M/s 2-1 α , leu ade-1 and M/s 2-2 *a* leu-1 thr2-1; originating from the cross 55R5/3c *a* ural $\times$ s2615B α thr2-1 tyr4, leu1, ade1, ade5. The strain 55R5 was obtained from Professor P. Slonimski, Gif-sur-Yvette, France, and strain s2615B from Professor R. K. Mortimer, Donner Laboratory, University of California, Berkeley, U.S.A.

Media. For yeast culture YPG medium was used consisting of 1 % Difco yeast extract, 1 % Difco-Bacto-Peptone and 2 % glucose. For DNA synthesis assay a medium consisting of 0.2 % yeast extract, 0.8 % Bacto-peptone and 3 % glucose, pH 4.5 was used. For estimation of the number of α - and *a*-cells a minimal medium was used (Glazy & Slonimski, 1957) with 2 % glucose and enriched as required with leucine and adenine or with leucine, threonine and methionine.

Microscopical examination. The cells were counted in a haemocytometer under a light microscope. For estimation of synchronization, cells with and without buds were counted as single units and the ratio of budded cells to the total number of cells was calculated. The cells were sonicated twice for 30 s, amplitude 2 μ m, with an MSE 100 watt ultrasonic disintegrator, then cooled on ice and immediately counted.

Culture. Both haploid strains were cultivated in 350 ml Erlenmeyer flasks containing 100 ml of YPG medium for 18 h at 30°C on an orbital shaker (120 rev./min). The concentration of cells in inocula was 3×10^5 for the *a*-strain and 2×10^6 for the α -strain. Cells were harvested at the beginning of the stationary phase.

Conjugation. The procedure of Biliński *et al.* (1973) was used.

Determination of DNA synthesis. This was carried out according to Hatzfeld (1973).

Measurements of DNA radioactivity. Samples (0.2 ml) containing DNA hydrolysates were mixed with 5 ml methanol and 10 ml scintillation liquid [2, 5-diphenylonazole (PPO), 13 g, and 1,4-di-1,2,5-phenylonazolyl-1-benzene (POPOP), 162.5 mg, in 1 l Packard scintillation grade toluene]. The radioactivity was assayed in a Liquid Scintillation Spectrometer Tricarb, Packard Model 3320.

Separation of yeast cells on Dowex 1 $\times$ 4 resin. Using the different abilities of various yeast strains to adsorb on Dowex 1 $\times$ 4 resin (Biliński & Litwińska, 1974) we separated the *a*- and α -cells from the conjugation mixture. The mixture of *a*- and α -cells (prepared as described in Fig. 2) was centrifuged, suspended in 3 mM-sodium acetate, sonicated and diluted to a final volume of 17 ml. The 16 ml samples of the suspension were added to flasks containing 5 g Dowex 1 $\times$ 4 in acetate form (washed with 70 % ethanol and sterilized water and decanted). The flasks were incubated for 60 min on an orbital shaker. After incubation the contents were poured on to 2 $\times$ 12 cm glass columns to separate the resin from the yeast suspension. The number of yeasts in the suspension collected under the columns was determined and the cells were plated on selective media to check the separation of mating types. Samples (10 ml) of this suspension were centrifuged and assayed for incorporation of radioactive adenine into DNA. The solutions and yeast suspensions were kept on ice. The separation of yeasts from the suspension of resin was done at 4 °C. Under these conditions the α -strain was not adsorbed to the resin while the *a*-strain was strongly bound. After separation the contamination of α -cells by *a*-cells was between 0.8 and 5.6 %.

RESULTS AND DISCUSSION

Inhibition of budding in conjugation mixture

Hartwell (1973) has shown that in conjugation mixtures nearly 85 % of the cells are synchronized and unbudded. Using our procedure we observed nearly 96 % of unbudded cells in the conjugation mixture which meant that the two mating types were reciprocally synchronized.

*DNA synthesis in the conjugation mixture of *a*- and α -cells*

Figure 1 shows the result of a typical experiment. The inhibition of DNA synthesis distinctly precedes the fall in the percentage of yeasts with buds. This is understandable, since Hartwell's (1971 *a, b*) work has shown that DNA replication covers only 40 % of the cell cycle, whereas buds start to form just after DNA synthesis initiation and separate only at the end of the cell cycle. In the present experiments stages of bud growth were not distinguished.

The inhibition of DNA replication in the conjugation mixture was quite conspicuous and suggests a mutual inhibition in both mating types. The residual level of incorporation of labelled adenine into DNA (about 20 % of the control level) may represent the level of adenine incorporation into mitochondrial DNA. Throm & Duntze (1970) suggested that the α -factor inhibits only nuclear DNA synthesis in *a*-cells.

Fig. 1. DNA replication and the percentage of budding cells in conjugation mixture of *a*- and α -yeasts. After 30 min adaptation to the medium the yeasts of both mating types were centrifuged and suspended in fresh medium at a density of 10^8 cells/ml. Yeasts of both mating types were mixed in a 1:1 ratio and also incubated separately. Samples (1 ml) were taken each 15 min and incubated with radioactive adenine (^3H -2, specific activity 17Ci/mM, The Radiochemical Centre, Amersham, Buckinghamshire) for the next 15 min. They were then diluted with 1 ml medium enriched with 1 mg of cold adenine/ml and cooled on ice immediately. The radioactive samples were precipitated with trichloroacetic acid and treated as in Methods. Non-radioactive samples were sonicated and after tenfold dilution the percentage of budded cells was determined. $\circ$, Budded α -cells; $\bullet$, budded *a*-cells; $\blacksquare$, budded cells in conjugation mixture; $\square$, radioactive adenine incorporation into DNA in conjugation mixture as compared to average incorporation in separately incubated suspensions of *a*- and α -yeasts. The differences in c.p.m. in DNA after 15 min of pulse in 10^8 cells at the beginning and at the end of incubation were as follows:

Strain	After 15 min	After 135 min
α	397	2710
<i>a</i>	346	2130
Mixture	370	481

Studies on budding and DNA synthesis in an α -strain separated from the conjugation mixture

As nothing is known about DNA inhibition in α -cells in a conjugation mixture with *a*-cells, we separated the two kinds of yeast from the mixture by the method of adsorption on a Dowex resin 1×4 (Biliński & Litwińska, 1974). Various yeast strains have different abilities to adsorb to the resins and this made possible a practically complete separation of the different components.

For comparison of DNA replication in α -cells in the mixture with that in *a*-cells and α -cells incubated alone in identical conditions, we followed incorporation of labelled adenine

Fig. 2. DNA replication and cell budding in α -cells isolated from conjugation mixture (○) and from a control non-conjugating mixture (●). Experimental conditions were as for Fig. 1. Samples were taken at 20 min intervals and 3 ml samples incubated for 20 min with 20 μ Ci radioactive adenine. After 20 min of incubation they were cooled on ice and diluted with 1 ml medium supplemented with 3 mg cold adenine/ml. A control mixture was obtained by mixing 2 ml of cooled suspensions of a - and α -cells after adenine incorporation. Both mixtures were then separated on resins, the radioactivity of DNA was measured and the number of budding cells counted.

(20 min pulse of radioactive adenine) in the conjugation mixture and in cultures of both mating types cultivated separately. To eliminate the possible influence of separation on resin columns, both strains were mixed after exposure to radioactive adenine and the same procedure of separation on resin was applied. The cells incubated separately showed continuous increase in the budding fraction during the period of incubation, whereas the α -cells from the conjugation mixture after a short period of increase showed a continuous fall in the fraction of budding cells (Fig. 2).

The inhibition of DNA replication in the α -strain was noticeable within the first 20 min, and after 60 min remained constant at 26 % of the control level. The results resemble those shown in Fig. 1 and suggest that the process of synchronization is very similar in both mating types.

Our results supported Hartwell's (1973) and Yanagishima's (1973) suggestions that a mutual synchronization of both a - and α -cells occurs in the conjugation mixture and that inhibition of DNA replication in α -cells during conjugation is parallel to that known already for a -type cells.

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