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Mating-Type-Dependent Inhibition of Deoxyribonucleic Acid Synthesis in *Saccharomyces cerevisiae*

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Yeast cells of mating type α excrete a sex factor which inhibits cell division and deoxyribonucleic acid replication but not ribonucleic acid or protein synthesis in cells of opposite mating type *a*.

The life cycle of the yeast *Saccharomyces cerevisiae* is controlled by the two alleles, *a* and α, of the mating-type gene located on chromosome III (6). Usually, diploid cells are heterozygous for the mating-type alleles (genotype a/α), whereas haploid cells contain only one of the two alleles (genotypes *a* or α) and, accordingly, exhibit phenotypically the corresponding mating type (7). Recently, it was found that cells of mating type α excrete a diffusible sex factor which inhibits the division of cells of the opposite (*a*) mating type (4). Because of its correlation with the α mating type, we termed this factor α factor. The affected *a* cells elongate, forming a tubelike process and, after prolonged exposure to the factor, develop into long bizarrely shaped forms that may reach 30 times the dry weight of normal haploid cells (W. Duntze and E. Throm, *unpublished data*). The excretion of α factor as well as the sensitivity of *a* cells to its action are properties that are strictly correlated with the corresponding mating types and, therefore, are thought to be genetically controlled by the mating-type alleles (4). We do not assume, however, that the α factor is the primary gene product of the α allele.

This note describes the effect of partially purified α factor on the synthesis of macromolecules in cells of different mating types. It was found that it specifically causes an inhibition of deoxyribonucleic acid (DNA) synthesis in cells of mating type *a*.

The diploid wild-type strain X2180 (a/α) and the isogenic haploid strains X2180-1A (*a*) and X2180-1B (α) derived from it by sporulation were used. The concentration and partial purification of α factor from culture filtrates of strain X2180-1B and the determination of the biological activity were described previously (4). One unit of activity per milliliter has been defined as the lowest concentration of α factor achieved by twofold serial dilutions that will induce the specific morphological reaction of *a* cells in the agar-diffusion assay described earlier (4).

The cells were grown aerobically at 30 C in MV medium (5) on a reciprocal shaker. α Factor was added to the cultures during the early exponential growth phase.

Figure 1 shows the influence of α factor on cell division, ribonucleic acid (RNA) synthesis, and DNA synthesis in a culture of *a* cells. After addition of 10 units of the factor per milliliter, the cells stop budding. DNA synthesis is inhibited, whereas RNA synthesis continues at the same rate as in the control culture for several hours. At this concentration of α factor, DNA synthesis is resumed and the elongated cells start to form new buds after 3 to 4 hr. Protein synthesis remains unchanged for several hours after addition of α factor (Fig. 2).

In cells of the isogenic strains X2180 (a/α) and X2180-1B (α), DNA synthesis and cell division are not affected by α factor (Fig. 3). The observed inhibition of DNA synthesis in *a* cells is not complete, however. Although the concentration of 50 units of α factor per ml totally inhibits cell division for several hours, there is still some incorporation of ^{14}C-adenine into deoxyribonuclease-sensitive material. Since the elongated *a* cells contain great numbers of mitochondria as revealed by electron microscopy (W. Duntze, *unpublished data*), this could be due to the replication of mitochondrial DNA.

Several conditions which specifically inhibit DNA synthesis without affecting protein and RNA synthesis have been found to lead to the formation of giant cells in bacteria (e.g., 1, 3). Furthermore, it was shown that replication of DNA is a necessary prerequisite for cell division

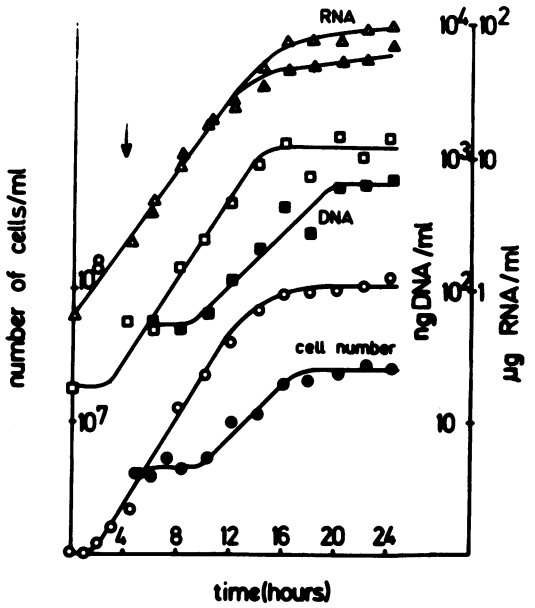

FIG. 1. *Inhibition of cell division and DNA synthesis in cells of mating type a (strain X2180-1A). Cells were grown in MV medium, and α factor was added, as indicated by the arrow, to one portion of the culture at a final concentration of 10 units/ml. Cell numbers were counted in a hemocytometer chamber. Total DNA was determined by the diphenylamine method and total RNA by the orcinol method described by Ogur et al. (8). Open symbols, control; closed symbols, culture containing α factor; ○, cell number per milliliter; □, nanograms of DNA/ml; △, micrograms of RNA per milliliter.*

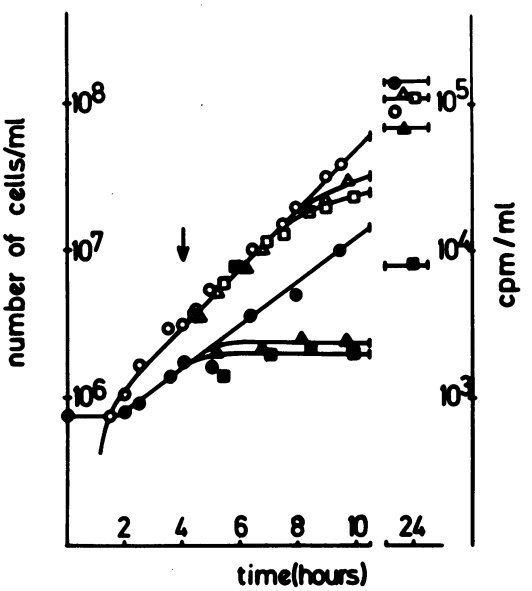

FIG. 2. *Incorporation of ^{14}C-leucine in the presence of α factor. Cells (strain X2180-1A) were grown in MV medium in the gresence of ^{14}C-leucine (0.23 µmoles/ml, 0.83 µCi/µmole). α Factor was added after 4 hr (arrow) at concentrations of 10 and 50 units/ml. Cells were precipitated with 5% trichloroacetic acid, incubated at 98 C for 30 min, collected on cellulose nitrate filters, washed, and counted in a liquid scintillation system. Open symbols, counts per minute of ^{14}C-leucine incorporated; closed symbols, cell number per milliliter; ○, control; △, 10 units of α factor/ml; □, 50 units of α factor/ml.*

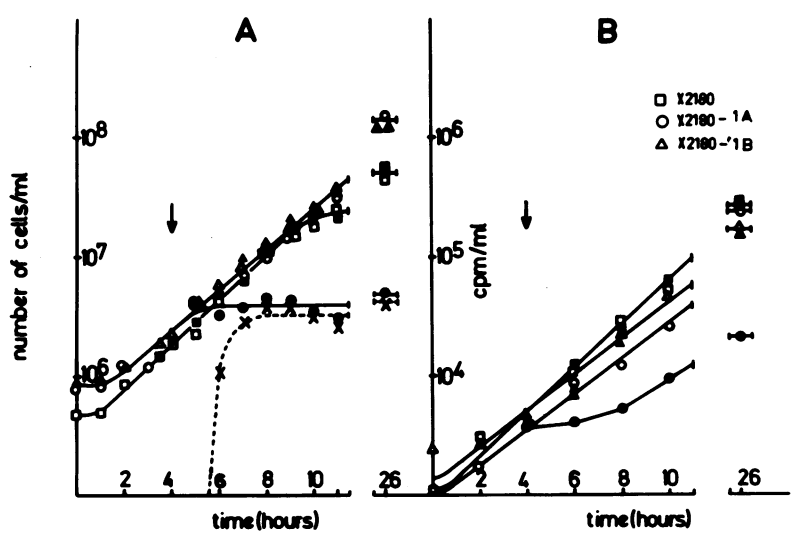

FIG. 3. *Specific inhibition of cell division (A) and DNA synthesis (B) of cells of mating type a by α factor. Cells of strains X2180 (□), X2180-1A (○), and X2180-1B (△) prelabeled with ^{14}C-adenine were grown in MV medium in the presence of ^{14}C-adenine (0.3 µmole/ml, 1.67 µCi/µmole. α Factor was added after 4 hr (arrow) at a concentration of 50 units/ml. Cell number as a function of time is plotted in A. Open symbols, control cultures; closed symbols, cultures containing α factor. For measurement of DNA, duplicate samples were hydrolyzed in 1 N NaOH at 25 C for 16 hr and then dialyzed overnight against 0.1 M tris(hydroxymethyl)aminomethane, pH 7.4, 0.03 M Mg^{2+}. Portions of each sample were either precipitated directly with 10% trichloroacetic acid or incubated for 4 hr with 80 µg of deoxyribonuclease per ml before precipitation. Deoxyribonuclease-sensitive counts per minute per milliliter of culture are plotted as a function of time in B. The appearance of morphologically altered cells in the culture of X2180-1A containing α factor is indicated by crosses (×).*

(2). Thus, it seems likely that the formation of giant forms in yeast cells of mating type *a* in the presence of α factor is due to its specific inhibitory effect on DNA synthesis. However, an additional influence of the factor on mechanisms involved in cell division cannot be excluded.

Although the genetic control of the mating-type system in *S. cerevisiae* is well established, the biochemical processes that are governed by the mating-type alleles and lead to the phenotypic differentiation of the two mating types are, so far, entirely unknown. The different properties of the two mating types described here which are amenable to biochemical investigation probably will help to understand the regulatory functions of these alleles on the molecular level.

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