Control of Dimorphism in *Candida albicans* by Zinc: Effect on Cell Morphology and Composition

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Zinc participates in the morphogenesis of a wide range of micro-organisms. In several dimorphic fungi such as *Histoplasma capsulatum* (Pine & Peacock, 1958), *Mucor rouxii* (Bartnicki-Garcia & Nickerson, 1962) and *Candida albicans* (Widra, 1964), zinc completely reverses the filamentous or mycelial phase of growth. Fungal cultures in which the morphology is influenced by a single factor can be useful for the study of metabolic processes underlying the dimorphic phase conversion and in this paper the changes in some macromolecular constituents of *C. albicans*, associated with its morphological transformation, are described with respect to the concentration of zinc added to the defined basal medium.

**METHODS**

*Candida albicans* strain 6713, which forms extensive filaments under some nutritional conditions (Yamaguchi, 1974a) was grown on a medium depleted of trace metals and containing (per l): glucose, 8 g; (NH₄)₂SO₄, 8 g; KH₂PO₄, 3·6 g; Na,HPO₄·12H₂O, 1·2 g; MgSO₄·7H₂O, 0·2 g; D-biotin, 0·1 µg. In zinc-replete medium ZnSO₄·7H₂O was added at 9 µM. Zinc-deficient medium was unsupplemented. Medium was inoculated (per ml) with about 10 µg dry wt of *C. albicans* taken from the surface of a 2-day-old culture grown at 37 °C on Sabouraud’s glucose agar slants, washed three times with double-distilled water, and finally resuspended in water.

Growth conditions and methods for assay of dry weight, the proportion of yeastlike-phase (Y) cells to filamentous-phase (F) cells during growth, and the content of DNA, RNA and protein were as previously described (Yamaguchi, 1974a). DNA phosphorus and RNA phosphorus were calculated by using the atomic extinction coefficient for phosphorus proposed by Logan, Mannell & Rossiter (1952). Insoluble inorganic polyphosphate in the hot 1 M-HClO₄ extract of the yeast was determined by adsorbing the nucleic acids on charcoal according to the method of Harold (1960), and the residual phosphorus, all of which was acid-labile, was taken as insoluble inorganic polyphosphate. After incinerating with conc. H₂SO₄ plus conc. HNO₃, the resulting orthophosphate was determined by the method of Fiske & SubbaRow (1925).

**RESULTS AND DISCUSSION**

The yield of *C. albicans* after 24 h growth was maximal with 3 µM-Zn²⁺; zinc above 10⁻³ M was inhibitory to growth (Fig. 1). Zinc-deficient cultures consisted almost entirely of F cells but the proportion of Y cells increased with increasing concentrations of zinc, reaching a maximal level at 9 µM-Zn²⁺ (Fig. 1). Observations with the light microscope agreed with these results, which were obtained using a mechanical filtration method.
Short communication

Fig. 1. Effect of different concentrations of zinc added to medium on total growth and morphology of Candida albicans. ●, Dry wt of yeast; ○, proportion of Y cells.

Table 1. Effect of zinc on the yield and concentrations of macromolecular constituents of Candida albicans during exponential and post-exponential growth

<table>
<thead>
<tr>
<th>Age of culture (h)</th>
<th>Yield (g dry wt/l)</th>
<th>Protein (µg)</th>
<th>DNA (µg P)</th>
<th>RNA (µg P)</th>
<th>Polyphosphate (µg P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Zn²⁺ added at 9 x 10⁻⁶ M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.12</td>
<td>292</td>
<td>0.32</td>
<td>2.84</td>
<td>12.8</td>
</tr>
<tr>
<td>8</td>
<td>0.59</td>
<td>290</td>
<td>0.17</td>
<td>3.00</td>
<td>12.3</td>
</tr>
<tr>
<td>12</td>
<td>1.92</td>
<td>312</td>
<td>0.18</td>
<td>3.00</td>
<td>12.1</td>
</tr>
<tr>
<td>18</td>
<td>3.01</td>
<td>376</td>
<td>0.16</td>
<td>2.62</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>3.13</td>
<td>405</td>
<td>0.19</td>
<td>2.53</td>
<td>12.0</td>
</tr>
</tbody>
</table>

No addition of Zn²⁺

| 4                  | 0.11               | 273          | 0.35       | 3.60       | 16.0                |
| 8                  | 0.51               | 306          | 0.27       | 2.43       | 19.1                |
| 12                 | 0.89               | 300          | 0.22       | 1.67       | 20.2                |
| 18                 | 1.75               | 309          | 0.21       | 0.79       | —                   |
| 24                 | 2.08               | 314          | 0.23       | 0.69       | 20.4                |

For the first 6 h of growth, zinc-deficient and zinc-replete cultures grew at the same rate with a doubling time of about 1.5 h. Subsequently, zinc-replete cultures still grew exponentially for about 5 h before slowing down, whereas the growth rate of zinc-deficient cultures dropped to about half but growth continued almost linearly at this rate for another 15 h. Growth of the deficient cultures over the latter period consisted essentially of filament elongation. It continued for at least 24 h after the yeast had reached its maximum dry weight.

Changes in content of several macromolecules were examined in both zinc-replete and zinc-deficient cultures (Table 1). There was little difference between the two cultures in the protein and DNA contents throughout the growth phase. The concentrations of RNA and inorganic polyphosphate did not change significantly when there was an adequate concentration of zinc, but when zinc was deficient the RNA content fell to a third and the content of inorganic polyphosphate doubled during the post-exponential phase of growth. The
inhibition of RNA synthesis and the accumulation of polyphosphate in the zinc-deficient cultures were apparent before inhibition of growth was observed.

The present data suggest that zinc has a primary function in metabolism of RNA in *C. albicans*, rather than protein or DNA. Zinc may be required for the synthesis of RNA or in preventing its degradation. Whatever the mechanism of action may be, the complete cessation of net RNA synthesis at the transitory stage from Y growth to F growth, under a certain degree of zinc deficiency, may imply that subsequent filamentous elongation takes place without net ribosomal formation. There is therefore a significant difference in the metabolic processes involved in mycelial elongation (F growth) and simple cell division (Y growth). Low RNA concentrations and a high incidence of F forms also occur when *C. albicans* is grown with an insufficient supply of yeast extract (Yamaguchi, 1974a) or biotin (Yamaguchi, 1974b).

Polyphosphate probably functions as store of 'high-energy' phosphate, as it may participate in the reversible synthesis of ADP and ATP (Yoshida & Yamataka, 1953). The accumulation of polyphosphate in zinc-deficient cultures of *C. albicans*, therefore, suggests that cessation of both net RNA synthesis and growth is not due to a failure of energy-yielding metabolism. There may be an antagonism between polyphosphate and nucleic acid metabolism, each competing for ATP, as has been postulated by Smith, Wilkinson & Duguid (1954) and Mudd, Yoshida & Koike (1958). The physiological role of such metabolism of phosphorous compounds in the F growth is being investigated.

I am grateful to Professor K. Iwata for valuable advice and to Miss Akiko Sawanobori for excellent technical assistance.

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