The anti-oestrogen drug tamoxifen (one of a series of synthetic triphenylethylene compounds) is widely used in the chemotherapy of breast cancer. Yeast is particularly interesting as it may be that conversion of the drug to an active form is believed to be due to binding to the oestrogen receptor, but there is also evidence for the existence of one or more additional mechanisms for tamoxifen activity [EBP] [41.]

The radioactive precursors listed were cultured for 24 h with rat colonic tissue. Soluble fractions were measured as the Vt fraction after Sepharose CL 4B chromatography and expressed relative to the DNA content of the tissue in culture.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Specific radioactivity (GBq/mmol)</th>
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<th>Soluble mucin (kBq/mg of DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>uL[3H]Fucose</td>
<td>3193</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>wL[3H]Mannose</td>
<td>603</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>uL[14C]Glucosamine</td>
<td>2.2</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Sodium [14C]acetate</td>
<td>1.9</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium [14C]butyrate</td>
<td>2.0</td>
<td>0.9</td>
<td>2.2</td>
</tr>
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This work was supported by the Medical Research Council, B.A.T. and the Glaxo Miles Trust.


Received 12 June 1989

### Table 1. Incorporation of precursors into mucin fractions in organ culture

The radioactive precursors listed were cultured for 24 h with rat colonic tissue. Soluble fractions were measured as the Vt fraction after Sepharose CL 4B chromatography and expressed relative to the DNA content of the tissue in culture.

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Received 12 June 1989

### Observation and significance of growth inhibition of Saccharomyces cerevisiae (A224A) by the anti-oestrogen drug tamoxifen

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The anti-oestrogen drug tamoxifen (one of a series of synthetic triphenylethylene compounds) is widely used in the chemotherapy of breast cancer [1]. Its major anti-tumour activity is believed to be due to binding to the oestrogen receptor, but there is already evidence for the existence of one or more additional mechanisms [2]. Recently, tamoxifen has been reported to inhibit translation in the rabbit reticulocyte system [3] and the significance of protein synthesis inhibition in the anti-tumour activity of tamoxifen requires further study.

We now report preliminary investigations on the yeast Saccharomyces cerevisiae, as a useful model in which to distinguish between several possible effects of tamoxifen on eukaryotic cell growth. Yeast is particularly interesting as it has been reported to possess an oestrogen-binding protein [EBP] [4].

Saccharomyces cerevisiae strain A224A grown overnight in YEPD medium [1% (w/v) yeast extract, 2% (w/v) Bactopeptone and 2% (w/v) glucose]; EBP, oestrogen-binding protein.

Abbreviations used: YEPD medium [1% (w/v) yeast extract, 2% (w/v) Bactopeptone and 2% (w/v) glucose]; EBP, oestrogen-binding protein.

Peptone and 2% (w/v) glucose] at 30°C in an orbital shaker (220 rev./min) was used to inoculate YEPD medium to an initial A600 of approx. 0.1. Tamoxifen was then added (from a series of freshly prepared stock solutions in 100% ethanol) to 20 ml portions of yeast culture in logarithmic phase growth at an A600 of 0.2 in 100 ml flasks shaken at 220 rev./min at 30°C, to give four final concentrations in the range 3.25–30 μM (at a final concentration of ethanol of 1% (v/v)).

Control experiments using 1% (v/v) ethanol were always included and, where 17-β-oestradiol was used, additions were made simultaneously with the tamoxifen, in equimolar amounts, each contributing an ethanol concentration of 0.5% (v/v) to the final concentration of 1% (v/v) ethanol.

Growth in the absence and presence of tamoxifen (with or without 17-β-oestradiol) was followed spectrophotometrically, with sampling every 30 min for 4.5 h.

Fig. 1 shows that tamoxifen inhibited the growth of A224A in a dose-dependent manner. At the tamoxifen concentrations used (3.25, 7.5, 15 and 30 μM) the inhibition calculated at 210 min after the addition of drug, which corresponds to 1.5 × generation time of the control containing 1% (v/v) ethanol, was 20, 45, 63 and 80%, respectively.

After the addition of the drug the onset of growth inhibition was detectable by this method after about 60 min. At this time the viability of the cells was markedly reduced by the two highest tamoxifen doses used (data not shown). It may be that conversion of the drug to a more active form is
Serine hydroxymethyltransferase activity during the growth of MOLT-4 cells in culture

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Serine has been classified as a nutritionally non-essential amino acid in animals because it can be endogenously synthesized from the glycolytic intermediate 3-phosphoglycerate. However, for the rapid proliferation of cells grown in culture, serine becomes a conditionally essential amino acid, presumably due to its ability to provide precursors for nucleotide biosynthesis de novo via the reaction catalysed by serine hydroxymethyltransferase (SHMT). This enzyme effects the transfer of a one-carbon moiety from serine to tetrahydrofolate, forming 5,10-methylenetetrahydrofolate with the concomitant formation of glycine. Methylenetetrahydrofolate is used directly for pyrimidine biosynthesis or alternatively 1% (v/v) di-methylsulphoxide, although a lower level of inhibition was observed due to the solvent alone, growth inhibition by tamoxifen was not significantly different to experiments in 1% (v/v) ethanol. Therefore we conclude that growth inhibition by tamoxifen is not affected by the presence of ethanol in the culture medium.

The observation that tamoxifen greatly inhibits the growth of S. cerevisiae A224A in a dose-dependent manner is of particular importance in view of the recently proposed long-term administration of tamoxifen as a prophylactic treatment for breast cancer because it suggests that this drug may have a range of adverse effects in cell growth that should be investigated.

The lack of response to 17-β-oestradiol is of special interest because EBP has been isolated and characterized from yeast and this EBP has a high affinity for oestrogens. However, the anti-oestrogens nafoxidine and tamoxifen, are known to show low affinity for the yeast EBP. The binding specificity profile for the Saccharomyces EBP is unique and quite unlike that of the mammalian oestrogen receptor [4].

Further investigations in vivo and in vitro are required to determine the mode(s) of inhibitory action of tamoxifen against yeast cells and these will involve the investigation of eukaryotic translation systems including that from Saccharomyces cerevisiae.


Received 9 June 1989

Fig. 1. Dose-dependency of inhibition of growth of Saccaromyces cerevisiae (A224A) by tamoxifen

A224A was grown in YEPD medium at 30°C, and at an A₅₆₅ₐ₅₀ of 0.2 the anti-oestrogen drug tamoxifen was added (with or without an equimolar amount of 17-β-oestradiol) to give the final drug concentrations 3.25 μM (●), 7.5 μM (△), 15 μM (■) and 30 μM (□), all with a final concentration of ethanol of 1% (v/v) [a control containing 1% (v/v) ethanol (□)] also included together with an untreated control (○). The presence of the 17-β-oestradiol did not significantly affect the growth inhibition levels due to tamoxifen (these superimposed points are not shown).