An Estrogen-Binding Protein and Endogenous Ligand in *Saccharomyces cerevisiae:* Possible Hormone Receptor System

Abstract. *A protein macromolecule in the cytosol of the unicellular eukaryotic yeast* Saccharomyces cerevisiae *selectively binds the vertebrate estrogen hormone 17β-estradiol with high affinity. Lipid extracts of the yeast cells or the conditioned growth medium yield a substance that can bind competitively to the tritiated estradiol–binding sites in the yeast and to mammalian estrogen receptors. These findings suggest that the binding protein may be a primitive hormone receptor and that the lipid-extractable substance represents the endogenous ligand.*

Messenger molecules in simple eukaryotic fungi may play a role analogous to that of pheromones and hormones in higher organisms (*1*). Peptide and sterol factors have been isolated and shown to play a role in mating-type conjugation (*2*), mycotoxins that exhibit estrogenic activity have been identified (*3*), a preliminary report finds that yeast possesses and can metabolize vitamin D hormonal metabolites (*4*), and the presence of immunoreactive hormone-like peptides has been reported in various simple organisms (*5*).

We recently described the existence of a corticosteroid binding protein and the possibility of an endogenous ligand in *Candida albicans* and postulated that a primitive steroid receptor system may be present in that pathogenic fungus (*6*). We have examined a variety of other fungi for the presence of vertebrate steroid hormone–binding proteins. In this study we scrutinized *Saccharomyces cerevisiae* because of its extensive use in molecular biology and genetics as well as its industrial and commercial importance. Although a corticosterone binding protein was not found, *S. cerevisiae* was shown to possess an estrogen-binding protein of high affinity and specificity. Furthermore, chloroform extracts of the organism contain a natural product that has the ability to bind competitively to the [^{3}H]estradiol binding sites present in *S. cerevisiae* and to estrogen receptors from rat uterus. The extractable substance, which appears to be the endogenous ligand for the *S. cerevisiae* binding protein, can be found in the yeast and can be recovered from the conditioned growth medium. We therefore postulate that *S. cerevisiae* possesses both components of a hormone-receptor system. We speculate that, in addition to the peptide factors known to play a role in the life cycle of this organism, a steroid-like hormone may also be active. Furthermore, the ability of the putative endogenous ligand from *S. cerevisiae* to interact with mammalian receptor sites suggests that this yeast product may have estrogenic effects in higher organisms.

Saccharomyces cerevisiae X2180-1B (α mating type) were grown in Yeast Nitrogen Base medium (6.7 g/liter; Difco) supplemented with glucose (20 g/liter). Cells were harvested late in logarithmic phase, washed, and disrupted with glass beads in an ice water–jacketed Bead Beater (Biospec Products). Cytosol prepared by ultracentrifugation was incubated with 17β-[6,7-^{3}H]estradiol (58 Ci/mmole; Amersham) for 2.5 hours at 25°C. Protein-bound hormone was separated from free hormone by G-50 Sephadex chromatography (*6*), and protein was measured by the Coomassie blue dye method (*7*).

In initial experiments a series of radioactively labeled steroids was used to screen *S. cerevisiae* cytosol for steroid-binding sites. No specific binding was detected following incubation at 0°C for 3 hours with [^{3}H]corticosterone, [^{3}H]dexamethasone, [^{3}H]testosterone, and [^{3}H]progesterone, all at a concentration of 26 n*M*, and with tritiated 1,25-dihidroxyvitamin D_3 (2.6 n*M*). However, incubation with [^{3}H]estradiol yielded abundant specific binding (*8*).

The binding site was found to be a 4.3*S* macromolecule on sucrose density gradients. The binding activity was destroyed by incubation with trypsin but not with deoxyribonuclease, ribonuclease, or phospholipase A_2, affirming the proteinaceous nature of the binding site. The [^{3}H]estradiol binding was reversible, and extraction and high-pressure liquid chro-

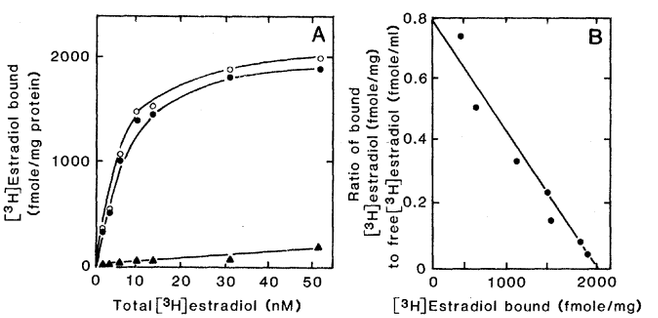

Fig. 1. Binding of [^{3}H]-estradiol to *S. cerevisiae* cytosol. Approximately 2 g (wet weight) of yeast cells were disrupted in a chilled Bead Beater in 20 ml of buffer containing 10 m*M* sodium phosphate, 1.5 m*M* EDTA, and 12 m*M* monothioglycerol (*p*H 6.4). Cytosol was prepared and incubated for 2.5 hours at 25°C with [^{3}H]estradiol (1.3 to 52 n*M*). Protein-bound hormone was separated from free hormone by G-50 Sephadex minicolumn chromatography. (A) Graph showing results of saturation analysis. Symbols: (○) total binding, (▲) nonspecific binding in the presence of 250-fold excess radioactively inert estradiol, and (●) specific binding, calculated by subtracting nonspecific from total binding. (B) Scatchard plot of specific binding data. The apparent equilibrium dissociation constant is 2.6 n*M* and the maximum number of binding sites is 2000 fmole per milligram of protein.

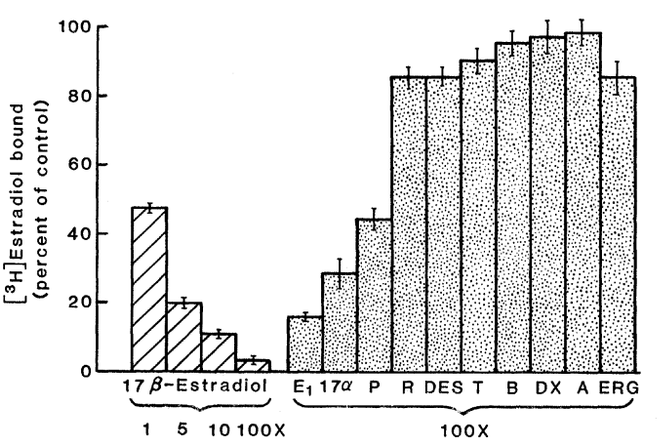

Fig. 2. Competition of a variety of steroids for [^{3}H]estradiol binding sites in yeast cytosol. [^{3}H]Estradiol (26 n*M*) was bound to cytosol at 25°C for 2.5 hours in the presence of various concentration ratios of estradiol (1, 5, 10, and 100× represent the molar ratio of the competitor to [^{3}H]estradiol). Other competing steroids were added at 2.6 μ*M* or a molar ratio of 100×. Binding in the absence of competitors (100 percent) averaged 984 ± 290 fmole per milligram of protein. The apparent activity of progesterone at 100× (2.6 μ*M*) should be judged by its rough equivalence to that of 1 × 17β-estradiol (26 n*M*), indicating a potency ~ 1 percent that of 17β-estradiol. Abbreviations: E_1, estrone; 17α-estradiol; *P*, progesterone; *R*, R-5020 (promegestone); *DES*, diethylstilbestrol; *T*, testosterone; *B*, corticosterone; *DX*, dexamethasone; *A*, aldosterone; *ERG*, ergosterol.

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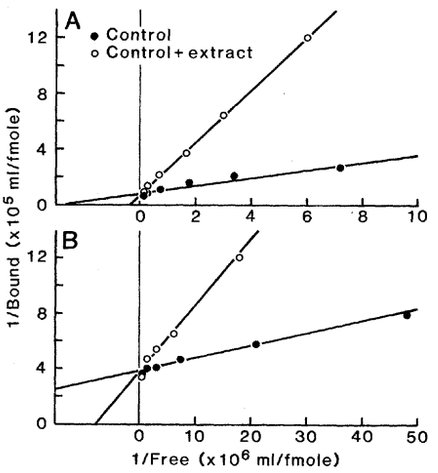

Fig. 3. Lineweaver-Burk analysis of the chloroform-extractable *S. cerevisiae* ligand, indicating competitive binding to two steroid-binding proteins. The *S. cerevisiae* were extracted by the method of Folch *et al.* (*9*), and the binding activity in the chloroform layer was fractionated over a silica gel column eluted sequentially with hexane, ethyl acetate, and methanol. Screening of all fractions revealed that most binding activity eluted in the area of the transition from ethyl acetate to methanol. The most active fractions were pooled, dried, resuspended in ethanol, and examined quantitatively for their ability to compete with [^{3}H]estradiol for specific binding. (A) Results for *S. cerevisiae* cytosol incubated with 1.3 to 52 n*M* [^{3}H]estradiol with and without yeast extract (equivalent to 100 mg wet weight of *S. cerevisiae* cells). (B) Results for ovariectomized rat uterine cytosol incubated with 0.26 to 13 n*M* [^{3}H]estradiol with and without yeast extract (same as panel A). Uteri were disrupted with a Polytron homogenizer in buffer containing 1.5 m*M* EDTA, 10 m*M* tris HCl, and 12 m*M* monothioglycerol (*p*H 7.8) and the cytosolic fraction was prepared. Specific binding was determined after 16 hours of incubation at 0°C by Sephadex chromatography.

matography of the bound [^{3}H]estradiol indicated that the bound moiety was unmetabolized hormone (*8*).

Figure 1A shows the results of a saturation analysis of the binding activity of α cell cytosol with [^{3}H]estradiol at 25°C for 2.5 hours. The concentration of [^{3}H]estradiol was varied from 1.3 to 52 n*M*; saturation of the binding sites occurred at ~ 25 n*M*. A plateau of approximately 2000 fmole of [^{3}H]estradiol bound per milligram of protein was attained. This value varied significantly in different experiments depending on culture conditions, suggesting regulation of the binding site concentration. Nonspecific binding constituted less than 10 percent of total binding. The specific binding data are replotted by the Scatchard method in Fig. 1B. The mean equilibrium dissociation constant measured in seven experiments was 3.0 ± 0.8 n*M* and the maximum number of binding sites was 1558 ± 200 fmole per milligram of protein.

The specificity of the binding site for steroids was also explored. Whereas estradiol was a potent competitor for [^{3}H]estradiol binding, the other steroid hormones tested showed variable ability to inhibit this binding reaction (Fig. 2). Estrone, a natural estrogenic molecule, exhibited 5 to 10 percent of the activity of estradiol. The relatively inactive stereoisomer 17α-estradiol was roughly 3 percent as potent as 17β-estradiol, the native vertebrate hormone. In contrast to the natural estrogens, diethylstilbestrol, a synthetic nonsteroidal estrogen, exhibited only minimal activity (< 1 percent). Progesterone was a weak competitor (< 1 percent), and R5020, a synthetic progestin, was even weaker. Testosterone, corticosterone, dexamethasone, aldosterone, and ergosterol all exhibited negligible competitive activity (< 1 percent of the potency of estradiol). The major difference between this profile and binding results with rat uterine estrogen receptors is the lack of activity of diethylstilbestrol in yeast and the fact that its potency is equivalent to that of 17β-estradiol in the mammalian receptor system.

Crude lipid extracts of the yeast cells or the conditioned growth medium yielded a substance that was able to compete for yeast cytosol [^{3}H]estradiol-binding sites in a dose-responsive manner. Various control experiments convincingly demonstrated that this material is produced by *S. cerevisiae* and that it is not an artifact of the extraction process. Figure 3A shows a Lineweaver-Burk analysis indicating that a partially purified preparation of the lipid extract can competitively inhibit [^{3}H]estradiol binding. Interestingly, putative endogenous ligand was also shown to be a competitive inhibitor of [^{3}H]estradiol binding to mammalian uterine estradiol receptors (Fig. 3B).

We speculate that the [^{3}H]estradiol-binding protein in *S. cerevisiae* represents a primitive estrogen receptor. In addition, the endogenous ligand may represent a hormone-like substance, possibly a steroid, that may mediate physiological effects in the yeast by binding to the [^{3}H]estradiol-binding protein.

Even if this hormone receptor hypothesis does not prove to be correct, the estrogen binding protein and the endogenous ligand are of great interest. The protein represents a new *S. cerevisiae* gene product of unknown function. The binding of the yeast ligand to mammalian estrogen receptors indicates the potential for a functional response, either agonistic or antagonistic, if sufficient ligand is present to occupy these sites.

That unicellular eukaryotic organisms may possess the elements of a steroid hormone receptor system raises several interesting issues. First, the emergence of a hormone system appears to have occurred much earlier in evolution than is generally thought. Second, the system seems to be highly conserved, judging from the substantial affinity exhibited by 17β-estradiol for the yeast binding protein and from the ability of the yeast ligand to bind to the mammalian receptor. Finally, the ready availability of genetic analysis and recombinant DNA techniques makes *S. cerevisiae* an ideal model system for further studies to elucidate the mechanism of steroid hormone action.

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References and Notes

1. T. Sandor and A. Z. Mehdi, in *Hormones and Evolution*, E. J. W. Barrington, Ed. (Academic Press, New York, 1979), vol. 1, pp. 1–72; G. Csaba, *Biol. Rev. Cambridge Phil. Soc.* **55**, 47 (1980); J. Roth, D. LeRoith, J. Shiloach, J. L. Rosenzweig, M. A. Lesniak, J. Havrankova, *N. Engl. J. Med.* **306**, 523 (1982).
2. A. W. Barksdale, *Science* **166**, 831 (1969); W. E. Timberlake, *Dev. Biol.* **51**, 202 (1976); R. Betz, T. R. Manney, W. Duntze, *Gamete Res.* **4**, 571 (1981).
3. B. S. Katzenellenbogen, J. A. Katzenellenbogen, D. Mordecai, *Endocrinology* **105**, 33 (1979); S. V. Pathre and C. J. Mirocha, in *Estrogens in the Environment: Mycotoxins as Estrogens*, J. A. McLachlan, Ed. (Elsevier/North-Holland, New York, 1979), pp. 265–278; W. Powell-Jones, S. Raeford, G. W. Lucier, *Mol. Pharmacol.* **20**, 35 (1981).
4. L. Hillman, S. Salmons, B. Fiore, paper presented at the Fifth Workshop on Vitamin D, Williamsburg, Va., 14 to 19 February 1982.
5. D. LeRoith, J. Shiloach, J. Roth, M. A. Lesniak, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6184 (1980).
6. D. S. Loose, D. J. Schurman, D. Feldman, *Nature (London)* **293**, 477 (1981); D. S. Loose and D. Feldman, *J. Biol. Chem.* **257**, 492 (1982).
7. M. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
8. Details of the optimization of the binding conditions and characterization of the protein are described by A. Burshell, P. Stathis, Y. Do, D. Loose, and D. Feldman (in preparation).
9. J. Folch, M. Lees, G. H. Sloane-Stanley, *J. Biol. Chem.* **226**, 497 (1957).
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