Receptor-mediated elevation of adenylate cyclase by luteinizing hormone in *Candida albicans*

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Human luteinizing hormone (hLH) and the GTP analogue guanosine 5'-O-(3-thio)triphosphate stimulated morphogenesis in the dimorphic fungal pathogen *Candida albicans*. hLH bound specifically to subcellular fractions from *C. albicans* and stimulated adenylate cyclase activity in *C. albicans* microsomes. We provide the first demonstration of guanine-nucleotide-binding proteins (G-proteins) in *C. albicans*, and propose that the stimulation of *C. albicans* morphogenesis by hLH is mediated by a receptor-coupled adenylate cyclase system involving G-proteins.

Introduction

In *Saccharomyces cerevisiae*, adenylate cyclase performs a key role in the response of cells to environmental signals. For example, when cells are starved of glucose they arrest in the G1 phase of the cell cycle. Addition of glucose to the medium causes a stimulation of adenylate cyclase activity, leading to an elevation in the level of intracellular cAMP. This in turn activates cAMP-dependent protein kinases, resulting in a signal to the cells to grow and divide (for a review, see Engelberg *et al.*, 1989). Although it is clear that guanine-nucleotide-binding proteins (G-proteins) are involved in the transduction of environmental signals to adenylate cyclase in *S. cerevisiae* (Matsumoto *et al.*, 1988), no receptor molecule which may respond to the environment and activate G-proteins has been discovered. Similarly, no potential ligand which might bind to such a receptor and activate the cyclase system has been identified.

Recently, we reported a stimulation of morphogenesis in the dimorphic, opportunistic fungal pathogen *Candida albicans* by human luteinizing hormone (hLH: Kinsman *et al.*, 1988). We have also detected specific binding

proteins for this hormone in the yeast form of *C. albicans* (Bramley *et al.*, 1990). In the present study we describe an elevation of adenylate cyclase activity following incubation of *C. albicans* microsomes with hLH. These results represent the first demonstration of a receptor-coupled adenylate cyclase activity in yeast. We propose that the stimulation of *C. albicans* growth and morphogenesis by hLH is mediated by this system, which may involve G-proteins analogous to those present in *S. cerevisiae* and mammalian cells.

Methods

Chemicals. All chemicals were purchased from Sigma, and were of the highest grade available. One international unit (IU) of hLH was equivalent to 0.2 µg of hormone.

Organism and culture conditions. *C. albicans* (serotype A; NCPF 3153) was used throughout and was maintained on Sabouraud's dextrose agar (Oxoid). Medium composition is indicated for each experiment.

Germination experiments. Sabouraud's dextrose broth (1 litre) was inoculated with 3×10^7 blastospores and incubated for 16 h at 37 °C with shaking (120 r.p.m.). Late exponential phase blastospores were harvested by centrifugation, washed twice with distilled water, used to inoculate Lee's synthetic medium (Lee *et al.*, 1975; pH 6.5; 1 ml contained 2×10^6 blastospores) and incubated with or without hLH or guanosine 5'-O-(3-thio)triphosphate (GTP γ S) for 2 h at 37 °C. Incubations were placed on ice and the percentage of yeast cells which had germinated was determined using a haemocytometer (germination was

Abbreviations: GDP β S, guanosine 5'-O-(2-thio)diphosphate; GTP γ S, guanosine 5'-O-(3-thio)triphosphate; hLH, human luteinizing hormone.

defined as germ tubes in excess of the maximum diameter of a blastospore).

Specific ^{125}I -labelled hLH binding. The preparation of microsomes (6.5 mg protein ml^{-1} ; 50 μl assayed) and cytosol (21 mg protein ml^{-1} ; 50 μl) from whole spheroplast lysates of late exponential phase cultures of *C. albicans*, the preparation of sheep corpus luteum homogenate (10 mg protein ml^{-1} ; 10 μl) and the measurement of specific ^{125}I -labelled hLH (100 000 c.p.m. ml^{-1} ; 100 Ci g^{-1} ; 3.7×10^{12} Bq g^{-1}) binding were as described previously (Bramley *et al.*, 1990). In parallel experiments with sheep corpus luteum homogenate, the rate of binding was found to be unrelated to protein content.

Microsomal adenylate cyclase activity. Microsomes (10 mg protein ml^{-1}) were prepared from whole spheroplast lysates of late exponential cultures as described previously (Bramley *et al.*, 1990), and adenylate cyclase was assayed by measuring the production of radiolabelled cAMP from [α - ^{32}P]ATP using the method of Salomon *et al.* (1974: method C) with the following modifications: samples (final volume 100 μl), with or without hLH, were buffered using 50 mM-PIPES, pH 6.5 (instead of Tris), contained, additionally, GTP (10 μM) and 3-isobutyl-1-methylxanthine (1 mM) and were incubated for 30 min at 37 °C.

Identification of guanine-nucleotide-binding proteins. Rat liver microsomes were prepared as described by Adams *et al.* (1985). *C. albicans* and *S. cerevisiae* cytosols and microsomes were prepared as described by Bramley *et al.* (1990). SDS-PAGE, followed by transfer to nitrocellulose, and subsequent interaction with [α - ^{32}P]GTP (Amersham: 1 $\mu\text{Ci ml}^{-1}$; 37 kBq ml^{-1} ; 3000 Ci mmol^{-1}) were as described by Lapetina & Reep (1987) with the following modifications: transfer blots were rinsed for 1 h in binding buffer (50 mM-Tris/HCl, 0.1% Triton X-100, 5 mM-MgCl₂, 0.1% bovine serum albumin, pH 7.5); protein molecular mass standards were visualized on nitrocellulose using amido black stain [0.05% amido black, 40% (v/v) methanol and 10% (v/v) acetic acid in distilled water]. [α - ^{32}P]GTP binding was visualized by autoradiography using Fuji RX X-ray film with an intensifying screen, at -70 °C.

Results

Effect of hLH and GTP γ S on germination

C. albicans is a dimorphic fungus, and hLH stimulated the germination of the mycelial growth form from blastospores grown in a defined medium (Fig. 1*a*). These results are in close agreement with those of Kinsman *et al.* (1988), who demonstrated that hLH promoted germination of blastospores grown in rat serum (10%, v/v, in saline). The non-hydrolysable GTP analogue GTP γ S also stimulated germination (Fig. 1*b*). Maximal stimulation occurred at a concentration of 1 μM -GTP γ S, the degree of stimulation decreasing markedly at 10 μM -GTP γ S.

Specific ^{125}I -labelled hLH binding to cytosolic and microsomal fractions

Specific hLH-binding proteins are present in significant amounts in both microsome and cytosol fractions of *C. albicans* cells (Fig. 2*a, b*; Bramley *et al.*, 1990). This

Fig. 1. Effect of hLH (*a*) and GTP γ S (*b*) on germ tube formation in *C. albicans*. For experimental details see Methods. A mean of at least five assessments was obtained. The experiment was repeated three times and the mean and SEM were determined. Significant differences were noted between hLH or GTP γ S incubations and the control as determined by Student's *t*-test: *, $P < 0.1$; **, $P < 0.02$; ***, $P < 0.001$.

situation is different to that noted in mammalian ovary or testis, where virtually all the hLH receptors are recovered in the microsomal fraction (Bramley, 1981). Interestingly, the interaction of hLH with the fungal binder occurred much more rapidly than the binding of the hormone to sheep corpus luteum receptors (Fig. 2*c*).

Effect of hLH on adenylate cyclase activity

hLH stimulated adenylate cyclase activity in *C. albicans* microsomes (Fig. 3). For example, enzyme activity was approximately $1.5 \times$ the control value following a 30 min incubation of microsomes (25 μg protein) with 10 IU hLH. Furthermore, under identical assay conditions but in the absence of hLH, adenylate cyclase activity was stimulated (2.07 ± 0.3 of basal enzyme activity; mean $\pm$ SD, three determinations) by the non-hydrolysable GTP analogue GTP γ S (100 μM) while guanosine 5'-*O*-(2-thio)diphosphate (GDP β S; 10 μM), a GDP analogue, inhibited enzyme activity (0.84 ± 0.04 of basal activity; mean $\pm$ SD, three determinations). Basal adeny-

Fig. 2. Time-course of specific ^{125}I -labelled hLH binding to *C. albicans* microsomes (a) and cytosol (b), and to sheep corpus luteum (c). At equilibrium, specific binding accounted for approximately 70% of total binding of hLH to *C. albicans* microsomes, and approximately 40% and 80% of total binding of hLH to *C. albicans* cytosol and corpus luteum homogenate, respectively. Points represent means $\pm$ range for triplicate estimates. The experiment was repeated three times with essentially the same results.

Fig. 3. Effect of hLH on microsomal adenylate cyclase activity. For experimental details see Methods. Significant differences were noted between hLH incubations and control [2.5 ± 0.17 pmol cAMP formed min^{-1} (mg protein) $^{-1}$; mean $\pm$ SD, three determinations] as determined by Student's *t*-test: *, $P < 0.02$; **, $P < 0.001$.

late cyclase activity was 2.5 ± 0.17 pmol cAMP formed min^{-1} (mg protein) $^{-1}$ (mean $\pm$ SD, three determinations). These results suggest that the basic properties of *C. albicans* adenylate cyclase are similar to those of hormone and neurotransmitter-stimulated enzymes in higher organisms (Gilman, 1984; Pace *et al.*, 1985; Rodbell, 1980; Schramm & Selinger, 1984). However, enzyme activity was not affected by the presence of NaF (10 mM), suggesting that the *C. albicans* cyclase is more closely related to the α -factor-inhibited adenylate cyclase of *Saccharomyces cerevisiae* in this regard (Liao & Thorner, 1980).

GTP-binding proteins in cytosolic and microsomal fractions

The distribution of low-molecular-mass G-proteins in rat liver microsomes, *C. albicans* microsomes, *C. albicans* cytosol and *S. cerevisiae* microsomes is illustrated in Fig. 4. The identification of a number of rat liver microsomal G-proteins in the range 23–27 kDa is in close agreement with previous reports of low-molecular-mass binding components in rat liver microsomes (Comerford & Dawson, 1989). A number of binding proteins of approximately 21–36 kDa were identified in *S. cerevisiae* microsomes. Similar results were obtained by Novick *et al.* (1988). The major binding species in *C. albicans* microsomes had a molecular mass of approximately 24 kDa. This protein appeared to be present at a lower concentration in the *C. albicans* cytosolic fraction. Preincubation of the nitrocellulose transfer blot with GTP (10 μM) for 30 min abolished binding of [α - ^{32}P]-GTP to all preparations (data not shown).

Fig. 4. Detection of GTP-binding proteins on nitrocellulose blots and determination of their molecular masses. Protein (100 µg) was electrophoresed and blotted onto nitrocellulose, and the blot incubated with [α - 32 P]GTP as described in Methods. The lanes on the autoradiograph show labelled proteins from: A, rat liver microsomes; B, *C. albicans* microsomes; C, *C. albicans* cytosol; and D, *S. cerevisiae* microsomes. The molecular masses of labelled proteins were calculated from standards running with the mobilities shown on the left.

Discussion

The transition from the yeast to mycelial growth form of *C. albicans* is thought to be important during pathogenesis, and the stimulation of germination by physiologically significant concentrations of hLH may contribute to recurrent bouts of candidosis in women with high levels of hLH in the vagina (Fig. 1*a*; Kinsman *et al.*, 1988). In mammals, LH plays a key role in the regulation of gonadal function, and this is achieved through the interaction of the hormone with specific receptors on target cells (Hunzicker-Dunn & Birnbaumer, 1985). hLH bound rapidly and specifically to *C. albicans* cytosolic and microsomal preparations (Fig. 2*a, b*) and it is possible that the hormonal stimulation of *C. albicans* morphogenesis involves an interaction of hLH with fungal binding proteins. The binding of LH to mammalian cell surface receptors is transmitted through the membrane to the interior of the cell by the stimulation of the adenylate cyclase complex, resulting in the enhanced formation of cAMP from ATP (Hunzicker-Dunn & Birnbaumer, 1985). Incubation of *C. albicans* microsomes with hLH stimulated microsomal adenylate

cyclase activity (Fig. 3), suggesting that a similar regulatory mechanism may function in this yeast.

The dimorphic nature of *C. albicans* has been widely exploited in fundamental studies of eukaryotic cell development (Soll, 1985). Interestingly, during these studies, elevated intracellular cAMP levels have been implicated in the regulation of morphogenesis in *C. albicans*, and our demonstration of the elevation of adenylate cyclase activity and therefore, presumably, intracellular cAMP levels following the stimulation of germination, is in keeping with the results of other workers in this field (for review, see Soll, 1985). More recently, Egidy *et al.* (1990) stimulated germination of *C. albicans* yeast cells using L-proline, and measured adenylate cyclase activity in permeabilized cells. They demonstrated a fall in cyclase activity prior to the emergence of germ tubes followed by a marked elevation in enzyme activity during germination. Our demonstration of a highly reproducible elevation of adenylate cyclase activity, in association with the stimulation of morphogenesis in *C. albicans*, provides us with a model for studying associated biochemical mechanisms involved in the transition from the yeast to the mycelial

growth form of this organism. In particular, it will be important to establish which enzyme activities are stimulated by increased intracellular cAMP levels resulting from elevated adenylate cyclase activity.

The regulation of mammalian adenylate cyclase following the specific interaction of a hormone with a cell surface receptor, is mediated through the enhancement of GDP/GTP exchange on a G protein. The spontaneous hydrolysis of GTP by G proteins ultimately causes cessation of their stimulation of the effector molecule (in this case adenylate cyclase). However, the GTP analogue GTP γ S is resistant to hydrolysis and causes essentially irreversible activation of G proteins. Interestingly, GTP γ S stimulated adenylate cyclase in *C. albicans* microsomes, although to a lesser extent than its stimulation of mammalian adenylate cyclase activity (Pace *et al.*, 1985). Furthermore, GTP γ S stimulated germination of *C. albicans* yeast cells (Fig. 1b). This result is intriguing in that it implies that if enhancement of germination involved the interaction of the nucleotide analogue with a G protein, then the GTP γ S molecule must have traversed both the cell wall and plasma membrane.

The results of the present study suggest a remarkable degree of evolutionary conservation of the receptor-mediated mechanism for the elevation of adenylate cyclase by luteinizing hormone. In mammals, the G-proteins that have been directly implicated in hormone signalling are all multi-subunit proteins composed of three types of subunit, designated α , β and γ (for review, see Morgan, 1989). Although those G-proteins linked to mating-pheromone receptors in *S. cerevisiae* are heterotrimeric, the *S. cerevisiae* t-proteins involved in the regulation of adenylate cyclase are members of the monomeric *RAS* family (Engelberg *et al.*, 1989; Matsumoto *et al.*, 1988; Wigler *et al.*, 1988). On the basis of the interaction of proteins on nitrocellulose blots with [32 P]GTP, we have identified a number of putative *C. albicans* G-proteins (Fig. 4). Bhullar & Haslam (1987) have noted that the nitrocellulose blot method used during the current investigation for the detection of G-proteins is not suitable for the identification of mammalian G-proteins of high molecular mass (approx. 40 kDa or more). Therefore, it is possible that similar high-molecular-mass G-proteins are also present in *C. albicans*. Our results represent the first demonstration of the presence of G-proteins in *C. albicans*, and the relationship between these proteins and the monomeric and heterotrimeric G-proteins of *S. cerevisiae* and mammals, will form the basis of future investigations.

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