Influence of Oestradiol on Protein Expression and Methionine Utilization during Morphogenesis of *Paracoccidioides brasiliensis*

By KARL V. CLEMONS,¹* DAVID FELDMAN² AND DAVID A. STEVENS¹

¹*Santa Clara Valley Medical Center and Institute for Medical Research, San Jose, California 95128, USA*

^{1,2}*Divisions of Infectious Diseases¹ and Endocrinology², Department of Medicine, Stanford University School of Medicine, Stanford, California 94305, USA*

(Received 3 January 1989; accepted 1 February 1989)

The temporal sequence of cytosolic protein expression during phase transition of *Paracoccidioides brasiliensis* was examined. Electrophoretic analysis of cytosol proteins by one-dimensional SDS-PAGE revealed numerous differences between the mycelial and yeast forms as well as alterations induced by 17β -oestradiol. Using either protein staining or fluorography of [35 S]methionine-labelled proteins 30 phase-specific bands were detected, 12 mycelial-associated bands (range 30 to 140 kDa) and 18 yeast-associated bands (range 22 to 127 kDa). In cells undergoing mycelial to yeast transition after a shift from 25 °C to 37 °C, the protein patterns showed a temporal progression toward the yeast profile with the accumulation of yeast bands prior to observable morphogenesis. Five novel protein bands (range 23 to 50 kDa) were detected by silver staining during transition. Treatment of temperature-shifted mycelial cultures with 2.6×10^{-7} M-oestradiol altered observed profiles; 4 of 12 mycelial-associated bands were maintained whereas the appearance of the 5 novel transition bands and 9 of 18 yeast-associated bands was blocked or delayed. Analysis of [35 S]methionine-labelled proteins revealed that oestradiol induced label uptake by mycelial cells, blocked the synthesis of a 92 kDa yeast-specific band 72 h into transition, and diminished label incorporation 120 h into transition. In conjunction with these steroid-induced alterations of protein expression, little or no morphological transformation occurred. These results support our hypothesis that, analogous to mammalian steroid receptor action, the functional responses of *P. brasiliensis* to oestradiol are related to regulation of protein expression, presumably mediated via a specific binding protein–ligand complex.

INTRODUCTION

Paracoccidioides brasiliensis is one of several medically important fungi exhibiting reversible thermal dimorphism (Kane, 1984; Kobayashi *et al.*, 1985; San-Blas & San-Blas, 1985). The transition from the saprophytic mycelial-form (M) into the parasitic yeast-form (Y), which is thought to be a requisite step in the establishment of primary infection (Restrepo, 1985), can be induced by a temperature shift from 25 °C to 37 °C (San-Blas & San-Blas, 1985). Several studies have detailed the critical effect of temperature and the temporal appearance of morphological transition forms (Carbonell & Rodriguez, 1965; Patino *et al.*, 1984; Salazar & Restrepo, 1984). Less well understood, however, are the metabolic events and regulatory mechanisms involved during the M to Y transition. It has been suggested that cAMP may play a regulatory role in the first hours of M to Y transition (Paris & Duran, 1985). Biochemically, alteration of cell wall

* Correspondence should be sent to: Dept of Medicine, Division of Infectious Diseases, Santa Clara Valley Medical Center, 751 South Bascom Avenue, San Jose, CA 95128, USA.

Abbreviations: E₂, 17β -oestradiol; Y, yeast; M, mycelial.

glucans from $\beta(1\rightarrow3)$ -glycosidic linkages (M-form) to $\alpha(1\rightarrow3)$ -glycosidic linkages (Y-form) occurs (Kanetsuna *et al.*, 1972; San-Blas & Vernet, 1977), as well as an apparent reduction in the amount of disulphide linkages. The latter may be associated with the increase of protein sulphide reductase activity in the Y-form (Kanetsuna *et al.*, 1972). Other investigators have demonstrated the M-form of *P. brasiliensis* to be prototrophic for organic sulphur and the Y-form to be auxotrophic, requiring cystine, cysteine or methionine, although the enzymic basis of the auxotrophy is unknown (Paris *et al.*, 1985).

In previous studies we have demonstrated that the vertebrate steroid hormone 17β -oestradiol (E_2) inhibits M to Y transition of *P. brasiliensis* in a dose-dependent manner (Restrepo *et al.*, 1984). In addition, *P. brasiliensis* possesses a cytosolic protein (EBP) that binds E_2 with high affinity and stereospecificity (Loose *et al.*, 1983; Stover *et al.*, 1986). Only those steroid hormones which exhibited affinity for EBP inhibited M to Y transition; E_2 had the highest affinity and the greatest inhibitory activity (Loose *et al.*, 1983; Restrepo *et al.*, 1984). The steroid specificity of this response led to the hypothesis that EBP acts as a receptor which modulates the behaviour of the organism during M to Y transition. To investigate the possibility that E_2 -mediated inhibition of *P. brasiliensis* M to Y transition is a result of altered protein synthesis, we examined the patterns of cytosolic proteins present during transition. We demonstrate several M- and Y-phase specific proteins, as well as E_2 -induced alterations in the synthesis of specific proteins. In addition, we demonstrate that M to Y transition results in alteration of methionine utilization and that E_2 exerts an influence on the control of this cellular function.

METHODS

Growth of organisms. *P. brasiliensis* (isolate Ber) was utilized throughout the study. Organisms were maintained by serial transfer on agar slopes of modified McVeigh-Morton medium (MVM) (Restrepo & Jimenez, 1980) and incubated at either 25 °C for M-form or 37 °C for Y-form maintenance. Mycelial culture filtrate was collected from MVM broth cultures of *P. brasiliensis* after 2–4 weeks of growth at 25 °C on a gyratory shaker at 150 r.p.m., filter sterilized and stored at 4 °C prior to use as a growth supplement. To prepare liquid cultures for phase-transition, 200 ml of fresh MVM supplemented with 100 ml of culture filtrate were inoculated with the M- or Y-form of *P. brasiliensis* as described previously (Stover *et al.*, 1986). Liquid cultures were grown for 8 d on a gyratory shaker at 150 r.p.m. at 25 °C (M-phase) or 37 °C (Y-phase). After the 8 d growth period, E_2 (Sterealoids, Wilton, NH, USA) (final concentration 2.6×10^{-7} M in 0.1% ethanol) or ethanol alone (final concentration 0.1%) was added to M-phase cultures. M to Y transition was induced in some of these cultures by increasing the incubation temperature from 25 °C to 37 °C, shaking at 150 r.p.m. over a period of 24 to 120 h after treatment. M-phase controls remained at 25 °C and 150 r.p.m. shaking for an additional 24 h after the addition of E_2 or ethanol prior to sample preparation. Y-phase controls remained at 37 °C throughout the period of incubation.

Sample preparation. At various times after the initiation of phase-transition, cultures were collected for analysis. In certain experiments the organisms from the 300 ml cultures were concentrated into 10 ml by centrifugation (1500 g, 15 min, 25 °C). These cultures were pulsed for the final 2 h of their incubation period (i.e. pulsed from 22 to 24 h, 70 to 72 h or 118 to 120 h) at the appropriate temperature with 3.7 MBq [35 S]methionine (1.5–18.5 GBq mmol $^{-1}$; Amersham) with or without the addition of 10 μ M unlabelled methionine. After the 2 h pulse, the cultures were diluted to 50 ml with sterile distilled water, harvested by centrifugation (1500 g, 15 min, 4 °C), washed once in ice-cold homogenization buffer [62.5 mM-Tris/HCl, 4 mM-EDTA, 4% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, pH 6.8] and resuspended in homogenization buffer (50:50, v/v). Cultures not radiolabelled were harvested directly by centrifugation and washed as described.

Cells were disrupted by vigorous agitation of the cell suspension with glass beads (0.45 mm) on a vortex mixer and cytosol collected from the broken cell suspensions as described previously (Stover *et al.*, 1986). Tubes were kept cold throughout the procedure by immersion in ice. Each cytosol sample was assayed for protein by the technique of Bradford (1976) and, when appropriate, assessed for radioactivity. All cytosols were stored at –70 °C until required for electrophoresis.

SDS-PAGE techniques. Samples were prepared for electrophoresis by the addition of 20% (w/v) SDS in homogenization buffer to make a final concentration of 2% (w/v) SDS and boiled for 5 min. Bromophenol blue (0.001%) served as the tracking dye. Samples, whose content was standardized by equivalence of protein concentration or c.p.m. of radioactivity, were loaded and electrophoresed through a SDS-PAGE gel, 9% running gel, 4.5% stacking gel (37.5:1, acrylamide/bis-acrylamide) essentially as described by Laemmli (1970).

Proteins were visualized on some gels by silver staining. Gels run to assess *de novo* protein synthesis were first stained with Coomassie Brilliant Blue R250 and further processed for fluorography as described by Bonner & Laskey (1974). Exposure to X-ray film (XOmat AR, Kodak) was done at –70 °C for a period of 7–21 d. Results were assessed visually for qualitative changes in banding patterns.

RESULTS

Comparison of cytosolic proteins from mycelial and yeast cells

Although the M- and Y-forms of *P. brasiliensis* are morphologically distinct, differences in cytosolic protein patterns have not been described previously. To assess whether differences in protein pattern existed between M- and Y-form, *P. brasiliensis* was grown in liquid culture at 25 °C (M-form) or 37 °C (Y-form), and the cytosols compared using one-dimensional SDS-polyacrylamide gels. Analysis of protein banding patterns was done qualitatively by visual examination. Only those differences observed in each of five silver stain experiments or two of three fluorography experiments were considered reproducible and are described. It should be noted that qualitative and quantitative band differences may be observed in the Figures which were not reproducible. These changes are not described.

Protein patterns visualized by silver staining indicated that each morphological form (M or Y) of *P. brasiliensis* was distinct (Fig. 1). Based on the conditions stated above, a total of 30 bands met these criteria and were considered to be reproducible. The M-form contained 12 bands not discernible in the Y-form, ranging from approximately 30 kDa to 140 kDa. The Y-form was observed to have 18 bands not present in the M-form, ranging from approximately 22 kDa to 127 kDa. Representative protein patterns are shown in Fig. 1, with only form-specific differences that are discerned easily in the reproduction indicated. In addition to the differences indicated in Fig. 1, M-form specific bands of 140 and 30.5 kDa and Y-form specific bands at 119, 108, 102, 93, 82, and 62 kDa were observed.

Differences in patterns of *de novo* synthesized cytoplasmic proteins were assessed by [³⁵S]-methionine incorporation. Preliminary experiments indicated that although the Y-form incorporated the label into many cytosolic proteins, a minimal amount of label was incorporated by M-form into M-form proteins. The addition of 10 µM unlabelled methionine to M cultures during the 2 h labelling period resulted in substantial [³⁵S]methionine incorporation into cytosolic proteins (Fig. 2, lane 1). The addition of unlabelled methionine to Y-form cultures did not alter their pattern of [³⁵S]methionine incorporation (data not shown). The banding patterns of [³⁵S]methionine labelled proteins were fairly similar to those presented in Fig. 1; 7 of 12 M-specific and 13 of 18 Y-specific bands were discernible. One M-form band at 30.5 kDa and one Y-form band at 108 kDa are not indicated in Fig. 2. Several M- and Y-specific bands detectable by silver staining were not detected by fluorography. However, the protein patterns of M- and Y-forms were distinct when compared by either method (Figs 1 and 2).

Comparison of cytosolic proteins of hormone-treated and control cells

The possible influence of E₂ treatment on protein patterns was assessed by one-dimensional SDS-PAGE of cytosolic proteins from hormone-treated and control cells at various times during the course of M- to Y-form transition. *P. brasiliensis* M- and Y-form transition was observed microscopically in liquid shaker cultures within 120 h after a temperature shift from 25 °C to 37 °C, but was 85% inhibited by concentration of 2.6×10^{-7} M-E₂.

Although protein patterns of M and Y controls were very consistent, temporal protein patterns during transition proved to be variable in the disappearance or appearance of form-specific bands. Because these results suggested asynchrony of the cells during the extended events of morphological transition, only those changes observed in at least three replicate experiments were considered reproducible.

There were no reproducible differences in total protein stained patterns between ethanol-treated and E₂-treated M cultures maintained for 24 h at 25 °C (Figs 3 and 4, lanes 1). However, after the temperature shift from 25 °C to 37 °C numerous changes occurred during the 120 h assay period of M- to Y-form transition. Ethanol-treated control cultures gradually acquired Y bands with the concurrent disappearance of M bands, their protein patterns becoming most yeast-like by 120 h of transition (Fig. 3, lane 4). These results correlated with the microscopic changes in morphology (data not shown). Morphological M to Y transition was blocked by E₂, and the temporal sequence of protein patterns was altered. After the temperature shift, the pattern of the M-form was maintained through 72 h and comparatively fewer Y-form bands had appeared after 120 h of transition in E₂-treated cells (Fig. 4, lanes 2–4). M-form bands of 123, 99,

Fig. 1

Fig. 2

Fig. 1. Comparison of proteins from the cytosol fractions of M and Y form of *P. brasiliensis*. Proteins (10 μ g per lane) were resolved by one-dimensional electrophoresis through 9% SDS-PAGE gels and silver stained. Lane 1 shows mycelial proteins with the estimated molecular mass in kDa of M-specific proteins on the left; lane 2 shows yeast proteins with the estimated molecular mass in kDa of Y-specific proteins at the right. Only those phase-specific proteins which are readily discernible in the photograph are indicated. Respective positions of molecular mass standards in kDa (phosphorylase *b*, 92.5; bovine serum albumin, 66.2; ovalbumin, 45; carbonic anhydrase, 31) are noted on the far left.

Fig. 2. Comparison of [35 S]methionine labelled proteins of M and Y cells. M phase proteins were labelled in the presence of excess radioinert methionine as described in Methods. Cells were labelled for 2 h prior to disruption and fractionation. Electrophoretic conditions were the same as described in Fig. 1. Gel lanes were loaded with the same number of c.p.m. (approximately 40000) and processed for fluorography. As in Fig. 1, only readily discerned phase-specific bands are indicated. Lane 1 shows mycelial proteins, with specific bands indicated at the near left; lane 2 shows yeast proteins with specific bands noted on the right. Molecular mass standards in kDa are indicated at the far left.

Fig. 3. Comparison of proteins from the cytosol fractions of untreated *P. brasiliensis* undergoing M to Y transition. Electrophoretic conditions were as described in Fig. 1. Proteins were visualized by silver staining. Lane 1, M-form cells incubated solely at 25 °C; lanes 2, 3 and 4 cells preincubated at 25 °C and switched to 37 °C for 24, 72 and 120 h, respectively. Lane 5, Y cells incubated only at 37 °C. Novel transition bands are indicated between lanes 3 and 4, with the estimated molecular mass in kDa. For reference, several M and Y-specific bands are indicated with the estimated molecular mass in kDa at the near left and right, respectively. Molecular masses in kDa of standards are indicated on the far left.

83 and 46 kDa were maintained, whereas Y-form bands of 102, 98, 93, 92, 78, 34, 31, 30 and 22 kDa were blocked or delayed in appearance as compared to controls. Novel transition bands of 50, 41, 37, 32 and 23 kDa were observed in the ethanol-treated controls by 24 h and/or 72 h, but had disappeared by 120 h of transition (Fig. 3, lanes 2–4). These bands were absent or delayed in appearance to 120 h of transition in E_2 -treated cells (Fig. 4, lanes 2–4). In addition, fewer total bands were present in E_2 -treated cells after 120 h of transition as compared to the respective control (Figs 3 and 4, lane 4).

Fig. 4. Comparison of proteins from the cytosol fractions of *E*₂-treated *P. brasiliensis* undergoing M to Y transition with M and Y controls. Proteins were resolved through 9% SDS-PAGE and silver stained. Lane 1, M-cells incubated solely at 25 °C, treated with *E*₂ for 24 h; lanes 2, 3, and 4, M-cells after 24, 72, and 120 h of *E*₂ treatment at 37 °C, respectively. Lane 5, Y-cells incubated at 37 °C only. Lanes 1–4 represent M cultures treated with *E*₂ (2.6×10^{-7} M), and lane 5 represents the Y control. Note the maintenance of the M-form profile at 24 and 72 h (lanes 2 and 3) and the decreased total number of bands by 120 h (lane 4), which demonstrates little similarity to the Y-form profile (lane 5). For reference, the molecular mass in kDa of the transition bands (between lanes 3 and 4) as well as selected M-specific (near left) and Y-specific (near right) bands are indicated. Molecular masses in kDa of standards are indicated on the far left.

Comparison of [³⁵S]methionine-labelled proteins

To assess the temporal sequence of proteins synthesized *de novo* by control and hormone-treated cells during M- to Y-form transition, cultures were pulsed with [³⁵S]methionine for 2 h prior to disruption and cytosol proteins visualized by fluorography of gels. Additional

Fig. 5. Comparison of [^{35}S]methionine-labelled protein fractions of hormone-treated and untreated *P. brasiliensis* undergoing M to Y transition. Labelling was done in the presence of radioinert methionine for lanes 1 and 2, and in the absence of radioinert methionine for lanes 3–9. Gels were run and analysed as in Fig. 2. Lane assignments are as follows: 1, M control; 2, M E_2 -treated; 3, 5, and 7, M controls grown at 37 °C for 24 h, 72 h and 120 h, respectively. Lanes 4, 6, and 8 are M treated with E_2 (2.6×10^{-7} M) grown at 37 °C for 24 h, 72 h and 120 h, respectively. Lane 9 is the yeast control. Note the absence of the 92 kDa band in lane 6 and few labelled bands in lane 8. The positions of selected M- and Y-specific bands with estimated molecular mass in kDa are indicated for reference at the near left and right, respectively. Molecular masses in kDa of standards are indicated at far left.

not by Y-form or M to Y transitional cultures. Profile differences were not apparent between control and E_2 -treated 25 °C M cells when labelled in the presence of excess radioinert methionine (Fig. 5, lanes 1 and 2). However, control and E_2 -treated 25 °C M cells labelled in the absence of excess methionine displayed dramatic pattern differences (Fig. 6, lanes 1 and 2). Whereas few if any labelled proteins were apparent from 25 °C M controls (Fig. 6, lane 1), 24 h of hormone treatment reproducibly enhanced [^{35}S]methionine uptake and incorporation into several cytosolic proteins by nontransforming 25 °C M cells (Fig. 6, lane 2).

Acquisition of Y-specific and disappearance of M-specific proteins were observed after 24 h of M to Y transition in both control and hormone-treated temperature-shifted cells (Fig. 5, lanes 3 and 4). By 72 h and 120 h of M to Y transition, E_2 treatment had resulted in detectable differences. Most notable was the reduction and probable absence of a Y-specific band (approximately 92 kDa) present in control M to Y transition cultures which was not observable in the profile of E_2 -treated cells (Figs 5 and 6, lanes 5 and 6). A second overall difference relating to the total number of labelled bands was observed in the comparative profiles after 120 h of M to Y transition (Fig. 5, lanes 7 and 8). As is evident, [^{35}S]methionine incorporation and presumably protein synthesis was reduced, with many fewer bands observable in E_2 -treated cells after 120 h of M to Y transition, whereas the control M to Y transition cultures exhibited

Fig. 6. Comparison of [35 S]methionine-labelled proteins in cytosol fractions of E_2 -treated and untreated *P. brasiliensis* undergoing M to Y transition. Labelling was done in the absence of radioinert methionine. Gels were prepared and analysed as in Fig. 2. Lane assignments 1–6 are the same as in Fig. 5. Note the effect of E_2 on label incorporation of M-form (lane 2) as compared to untreated control (lane 1). Absence of the 92 kDa Y-specific band in lane 6 is indicated as in Fig. 5. The fluorogram was intentionally over-exposed to enhance the bands in lane 2. Molecular masses in kDa of standards are indicated on the left.

incorporation and a Y protein synthesis profile (Fig. 5, lanes 7–9). In separate experiments, reduction in the number of bands present in E_2 -treated cells was noted by total protein staining (Fig. 4, lane 4).

These results indicated that the switch to utilization and incorporation of exogenous methionine occurs within 24 h of temperature increase to 37 °C (Fig. 6, lanes 1 and 3) and that the synthesis of Y proteins began 24 to 72 h before observable morphological changes occurred.

DISCUSSION

In the present investigation, the temporal sequence of protein expression by E_2 -treated and non-treated cells of *P. brasiliensis* was studied during M to Y transition. The results presented here are in accord with and extend previous studies on temperature-induced form-transition by *P. brasiliensis*. Further, these results indicate that not only can temperature induce differential protein expression in this organism, but that the mammalian steroid hormone, E_2 , exerts demonstrable effects on protein expression. Because the morphological form of *P. brasiliensis* appears to be influenced primarily by the temperature of incubation (Patino *et al.*, 1984; San-Blas & San-Blas, 1985), it would be attractive to postulate form-specific repression or

derepression of the synthesis of some cellular proteins. Results of the present study are in accord with this hypothesis in that some proteins were observed to be associated with only the M- or Y-form and that during transition the M pattern progressively changed to the Y pattern. In addition, the appearance of novel transition bands suggest that they are involved in the morphological transition itself. The inconsistency of appearance of these bands, and the lack of detection by [35 S]methionine labelling, may be due in part to the relative asynchrony of these multinuclear fungal cells during the extended event of transition in combination with the short 'labelling window' utilized. Similarly novel transition proteins have been reported to occur during form-transition of *Candida albicans* and were designated as heat-shock proteins (Dabrowa & Howard, 1984). Whether or not the transition proteins observed in this study of *P. brasiliensis* are true heat-shock-type proteins as expressed by other prokaryotes and eukaryotes (Neidhardt *et al.*, 1984) remains to be determined.

Lambowitz *et al.* (1983), in studies with *Histoplasma capsulatum*, have proposed that dimorphism may be a byproduct of the heat shock response, occurring over a period of days with marked changes to oxidative phosphorylation and a switch to cysteine auxotrophy after shifting the temperature from 25 °C to 37 °C (Maresca *et al.*, 1981; Medoff *et al.*, 1987*a*). Likewise, a switch to Y-form auxotrophy for sulphur-containing amino acids (Paris *et al.*, 1985) and the uncoupling of oxidative phosphorylation as well as a cysteine requirement during transition (Medoff *et al.*, 1987*b*) has been described for *P. brasiliensis*. In accord, our results clearly demonstrate increased translocation and incorporation of [35 S]methionine by Y-form cells, whereas M-form cells incorporated little if any label. This change indicates increased utilization and possible growth requirement or auxotrophy for methionine in the Y-form. However, incorporation of [35 S]methionine by the M-form could be enhanced by increasing the extracellular concentration of methionine and appears to indicate a down-regulated, but fully functional, methionine translocation system. It is of interest that [35 S]methionine was incorporated well by cells 24 h after the initiation of transition. This change might be a result of diminished cellular capacity to synthesize methionine with subsequent up-regulation of a translocation system occurring to provide the cells with required methionine. These results are similar to those with *H. capsulatum* (Maresca *et al.*, 1981) and demonstrate that a switch in methionine metabolism by *P. brasiliensis* occurs several days before visible morphogenesis.

In conjunction with these temperature-related changes, the potential influence of E_2 on cellular protein patterns during M to Y transition was examined. Classically, steroid hormone action in mammalian systems is mediated via a receptor–ligand–DNA complex which regulates the expression of specific proteins (Yamamoto, 1985). In some fungi, mating pheromones regulate sexual reproduction and induce alterations in protein expression. Tremmerogen A-10 and a-13 induce expression of cell-surface proteins in the gametes of *Tremella mesenterica* (Miyakawa *et al.*, 1984). Antheridiol, secreted by female cells of *Achlya ambisexualis*, induces protein expression in male cells (Brunt & Silver, 1986*a, b*, 1987) which is regulated via a specific antheridiol receptor (Riehl *et al.*, 1984; Riehl & Toft, 1984). Although sexual mating is not known to occur in *P. brasiliensis*, steroid hormones may influence the organism. The possibility exists that E_2 in combination with the increase in temperature may in some manner directly affect the functions of cellular components, such as microsomal enzymes, necessary for phase-transition. However, we have demonstrated the presence of EBP, a cytosolic protein (≈ 60 kDa) which specifically binds E_2 . Because of the specificity of the response we postulated that EBP, acting as a receptor for E_2 , mediates E_2 inhibition of M- to Y-phase transition (Loose *et al.*, 1983; Restrepo *et al.*, 1984; Stover *et al.*, 1986). In accord with this hypothesis, the present study indicates that E_2 alters protein expression in *P. brasiliensis* undergoing transition.

Although some differences in protein patterns were detectable by silver staining, alterations in *de novo* protein synthesis were the most dramatic. Unlike *Tremella* (Miyakawa *et al.*, 1984) and *Achlya* (Brunt & Silver, 1986*a, b*), where the specific pheromones primarily induce new proteins, E_2 (which blocks a morphological event) blocked or delayed the synthesis of numerous proteins. Of these, most notable were the absence of a 92 kDa Y-specific band and the almost total ablation of *de novo* protein synthesis 120 h into the transition process. Thus, these results indicate that the inhibition of M to Y transition by E_2 is linked to repression of protein

synthesis. Because previous studies have shown no effects on Y to M transition or Y-form growth (Loose *et al.*, 1983; Restrepo *et al.*, 1984) and because E₂-treated cells continued to incorporate methionine through 72 h of transition, we do not feel that the ablation of protein synthesis was caused by an immediate or direct toxicity of E₂. The mechanism and duration of these responses to E₂ remain to be determined.

An unexpected finding in this study was that E₂ exerted an effect on the M-form of *P. brasiliensis* not undergoing phase transition. While M-form organisms did not readily incorporate [³⁵S]methionine into cytosolic proteins, E₂ treatment of M-form resulted in label incorporation. Although paradoxical, the switch by M-form cells to an ability to translocate and incorporate methionine, a property associated with the Y-form, is the first reported metabolic response exhibited by M-form *P. brasiliensis* to the presence of E₂. While the regulatory basis of this change is not known, it is interesting to note the similarity between this response induced by E₂ and the normal switch in nontreated cells after an increase in temperature. Thus, it is possible that our results reflect induction by E₂ of a stress-type response by M-form *P. brasiliensis*, which is pleiotropic in effect.

The distinct protein patterns of M- and Y-form cells are suggestive of temperature-regulated protein expression in *P. brasiliensis*. Furthermore, several days prior to visible morphological changes during the M- to Y-phase transition, differential protein expression occurs in a temporal fashion which can be modulated by E₂-treatment.

This study was supported by Public Health Service grant AI-20409 from the National Institutes of Health and by National Science Foundation grant INT-8601654.

REFERENCES

- BONNER, W. M. & LASKEY, R. A. (1974). A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *European Journal of Biochemistry* **46**, 83–88.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annals of Biochemistry* **72**, 248–254.
- BRUNT, S. A. & SILVER, J. C. (1986a). Steroid hormone-induced changes in secreted proteins in the filamentous fungus *Achlya*. *Experimental Cell Research* **163**, 22–34.
- BRUNT, S. A. & SILVER, J. C. (1986b). Cellular localization of steroid hormone-regulated proteins during sexual development in *Achlya*. *Experimental Cell Research* **165**, 306–319.
- BRUNT, S. A. & SILVER, J. C. (1987). Steroid hormone-regulated basic proteins in *Achlya ambisexualis*. *Experimental Mycology* **11**, 65–69.
- CARBONELL, L. & RODRIGUEZ, J. (1965). Transformation of mycelial and yeast forms of *Paracoccidioides brasiliensis* in cultures and in experimental inoculations. *Journal of Bacteriology* **90**, 504–510.
- DABROWA, N. & HOWARD, D. H. (1984). Heat shock and heat stroke proteins observed during germination of the blastoconidia of *Candida albicans*. *Infection and Immunity* **44**, 537–539.
- KANE, J. (1984). Conversion of *Blastomyces dermatitidis* to the yeast form at 37 °C and 26 °C. *Journal of Clinical Microbiology* **20**, 594–596.
- KANETSUNA, F., CARBONELL, L. M., AZUMA, I. & YAMAMURA, Y. (1972). Biochemical studies on the thermal dimorphism of *Paracoccidioides brasiliensis*. *Journal of Bacteriology* **110**, 208–218.
- KOBAYASHI, G. S., MEDOFF, G., MARESCA, B., SACCO, M. & KUMAR, B. V. (1985). Studies on phase transitions in the dimorphic pathogen *Histoplasma capsulatum*. In *Fungal Dimorphism with Emphasis on Fungi Pathogenic for Humans*, pp. 69–91. Edited by P. J. Szanislo & J. L. Harris. New York: Plenum Press.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.
- LAMBOWITZ, A. M., KOBAYASHI, G. S., PAINTER, A. & MEDOFF, G. (1983). Possible relationship of morphogenesis in pathogenic fungus, *Histoplasma capsulatum*, to heat shock response. *Nature, London* **303**, 806–808.
- LOOSE, D. S., STOVER, E. P., RESTREPO, A., STEVENS, D. A. & FELDMAN, D. (1983). Estradiol binds to a receptor-like cytosol binding protein and initiates a biological response in *Paracoccidioides brasiliensis*. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 7659–7663.
- MARESCA, B., LAMBOWITZ, A. M., KUMAR, V. B., GRANT, G. A., KOBAYASHI, G. S. & MEDOFF, G. (1981). Role of cysteine in regulating morphogenesis and mitochondrial activity in the dimorphic fungus *Histoplasma capsulatum*. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 4596–4600.
- MEDOFF, G., KOBAYASHI, G. S., PAINTER, A. & TRAVIS, S. (1987a). Morphogenesis and pathogenicity of *Histoplasma capsulatum*. *Infection and Immunity* **55**, 1355–1358.
- MEDOFF, G., PAINTER, A. & KOBAYASHI, G. S. (1987b). Mycelial-to-yeast-phase transitions of the dimorphic fungi *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*. *Journal of Bacteriology* **169**, 4055–4060.
- MIYAKAWA, T., KADOTA, T., OKUBO, Y., HATANO, T., TSUCHIYA, E. & FUKUI, S. (1984). Mating pheromone-induced alteration of cell surface proteins in

- the heterobasidiomycetous yeast *Tremella mesenterica*. *Journal of Bacteriology* **158**, 814–819.
- NEIDHARDT, F. C., VAN BOGELEN, R. A. & VAUGHN, V. (1984). The genetics and regulation of heat-shock proteins. *Annual Review of Genetics* **18**, 295–329.
- PARIS, S. & DURAN, S. (1985). Cyclic adenosine 3',5'-monophosphate (cAMP) and dimorphism in the pathogenic fungus *Paracoccidioides brasiliensis*. *Mycopathologia* **92**, 115–120.
- PARIS, S., DURAN-GONZALEZ, S. & MARIAT, F. (1985). Nutritional studies on *Paracoccidioides brasiliensis*: the role of organic sulfur in dimorphism. *Sabouraudia* **23**, 85–92.
- PATINO, M. M., BURGAS, L. C. & RESTREPO, A. (1984). Effect of temperature on the mycelium to yeast transformation of *Paracoccidioides brasiliensis*. *Sabouraudia* **22**, 509–511.
- RESTREPO M. A. (1985). The ecology of *Paracoccidioides brasiliensis*: a puzzle still unsolved. *Sabouraudia* **23**, 323–334.
- RESTREPO, A. & JIMENEZ, B. E. (1980). Growth of *Paracoccidioides brasiliensis* yeast phase in a chemically defined culture medium. *Journal of Clinical Microbiology* **12**, 279–281.
- RESTREPO, A., SALAZAR, M. E., CANO, L. E., STOVER, E. P., FELDMAN, D. & STEVENS, D. A. (1984). Estrogens inhibit mycelium-to-yeast transformation in the fungus *Paracoccidioides brasiliensis*: implications for resistance of females to paracoccidioidomycosis. *Infection and Immunity* **46**, 346–353.
- RIEHL, R. M. & TOFT, D. O. (1984). Analysis of the steroid receptor of *Achlya ambisexualis*. *Journal of Biological Chemistry* **259**, 15324–15330.
- RIEHL, R. M., TOFT, D. O., MEYER, M. D., CARLSON, G. L. & MCMORRIS, T. C. (1984). Detection of a pheromone-binding protein in the aquatic fungus *Achlya ambisexualis*. *Experimental Cell Research* **153**, 544–549.
- SALAZAR, M. E. & RESTREPO, A. (1984). Morphogenesis of the mycelium to yeast transformation in *Paracoccidioides brasiliensis*. *Sabouraudia* **22**, 7–11.
- SAN-BLAS, F. & SAN-BLAS, G. (1985). *Paracoccidioides brasiliensis*. In *Fungal Dimorphism with Emphasis on Fungi Pathogenic for Humans*, pp. 93–120. Edited by P. J. Szaniszlo & J. L. Harris. New York: Plenum Press.
- SAN-BLAS, G. & VERNET, D. (1977). Induction of the synthesis of cell wall α 1,3-glucan in the yeastlike form of *Paracoccidioides brasiliensis* strain IVIC Pb9 by fetal calf serum. *Infection and Immunity* **15**, 897–902.
- STOVER, E. P., SCHAR, G., CLEMONS, K. V., STEVENS, D. A. & FELDMAN, D. (1986). Estradiol-binding proteins from mycelial and yeast-form cultures of *Paracoccidioides brasiliensis*. *Infection and Immunity* **51**, 199–203.
- YAMAMOTO, K. R. (1985). Steroid receptor regulated transcription of specific genes and gene networks. *Annual Review of Genetics* **19**, 209–252.