Protein Synthesis and Amino Acid Pool during Yeast–Mycelial Transition Induced by *N*-Acetyl-D-glucosamine in *Candida albicans*

By ANTONELLA TOROSANTUCCI,¹ LETIZIA ANGIOLELLA,¹
CARMELA FILESI² AND ANTONIO CASSONE^{1*}

¹ *Laboratorio di Batteriologia e Micologia Medica and* ² *Laboratorio di Alimenti,
Istituto Superiore di Sanità, Rome, Italy*

(Received 4 June 1984; revised 6 July 1984)

Protein synthesis at different stages of yeast–mycelial transition induced by *N*-acetyl-D-glucosamine in *Candida albicans* was evaluated by following incorporation of radioactive amino acids into the acid-insoluble cellular material. In passing from the early germ-tube formation (60–90 min) to the mature hyphal cell (240–270 min) there was a marked decrease in the capacity for protein synthesis. Apparently, this decrease was not due to a decreased amino acid uptake into the soluble cellular pool or to exhaustion of carbon/energy source in the inducing medium with consequent arrest of growth. Protein synthesis, however, did not decay when amino acids at high concentration were added to the medium fostering the yeast–mycelial transition and this effect was potentiated by glucose.

Analysis of the intracellular amino acid pool showed that both germ-tubes and hyphal cells were relatively depleted of several amino acids as compared to the yeast-form cells, whereas in the hyphae there was a higher concentration of glutamic acid/glutamine, the latter being the predominant component. These modulations in amino acid pool composition were not seen when yeasts were converted to hyphae in an amino acid-rich induction medium. This study emphasizes that yeast-form cells of *C. albicans* may efficiently convert to the mycelial form even under a progressively lowered rate of protein synthesis, and suggests that initiation of hyphal morphogenesis in the presence of *N*-acetyl-D-glucosamine is somehow separated from cellular growth.

INTRODUCTION

Dimorphism in fungi is defined as the reversible transition from a yeast to a mycelial habit of growth (Bartnicki-Garcia & McMorrough, 1971). This phenomenon is of special relevance in *Candida albicans* for at least two reasons. First, it is an important, though controversial, factor in the pathogenesis of candidosis which now occupies a major place among the opportunistic diseases (Odds, 1979). Second, it affords a convenient and simple model to study cellular morphogenesis in lower eukaryotes. Thus, there is a common belief that the unravelling of the mechanisms of transition may help both to understand and control the disease and to gain valuable information about fungal morphogenesis in general. For these reasons, yeast–mycelial transition in *C. albicans* is increasingly studied *in vitro* where, apart from the mandatory temperature in the range 33–40 °C, it can be induced by a variety of compounds and unrelated nutritional conditions (Odds, 1979).

In most cases, however, the complexity of these conditions makes it difficult to assess the mechanism(s) underlying the induction phenomenon. Consequently, efforts were made to find simple compounds which might trigger an efficient and reproducible transition, so as to understand the metabolic pattern(s) leading to specific, dimorphism-related events.

It has been suggested that induction of hyphal conversion with the simple acetylated amino sugar *N*-acetyl-D-glucosamine (GlcNAc) could be a very useful model for studying the

dimorphic transition (Simonetti *et al.*, 1974). Using this model, it has been shown recently that GlcNAc derepresses the synthesis of the enzymes of its catabolic pathway (Gopal *et al.*, 1982; Singh & Datta, 1979) but that GlcNAc catabolism is substantially unrelated to the GlcNAc property of inducing hyphal conversion (Mattia *et al.*, 1982; Shepherd *et al.*, 1980a) which, in line with a previous suggestion (Simonetti *et al.*, 1974), could occur by a receptor-signal mechanism (Shepherd & Sullivan, 1983).

Despite this recent information, the mechanism of GlcNAc-induced dimorphic transition in *C. albicans* remains poorly understood. What appears to be a significant aspect of this model is the 'divergence' between protein and polysaccharide content with the former markedly decreasing and the latter markedly increasing in the hyphae as compared to the yeast (Mattia *et al.*, 1982). In general, protein metabolism has not been thoroughly investigated throughout the dimorphic transition and the problem concerning the existence of form-specific proteins in *C. albicans* is very much debated and controversial (Ahrens *et al.*, 1983; Brown & Chaffin, 1981; Brummel & Soll, 1982; Manning & Mitchell, 1980).

This paper is mainly concerned with the evaluation of the changes in protein synthesis during yeast-mycelial conversion induced by GlcNAc. It also reports a quantitative determination of the cellular amino acid pool at different times of induction.

METHODS

Organisms and growth conditions. Two strains of *Candida albicans* were used throughout this study. Strain BP was isolated from a clinical specimen and identified by the established taxonomic criteria (Lodder, 1970). Strain 3153A was kindly given by Dr W. Whelan (Department of Biochemistry, University of Cambridge, UK). Both strains could be classified as 'high responders' in that they convert to hyphae in GlcNAc/saline medium without any pretreatment (Mattia *et al.*, 1982). They were routinely maintained in the yeast form on Winge solid medium at 28 °C (Mattia & Cassone, 1979). Unless otherwise stated, the experiments were done with strain BP of *C. albicans*. For growth on GlcNAc and/or glucose as single carbon source, the micro-organism was first grown at 28 °C for several generations in a basal saline medium composed of (NH₄)₂SO₄, 0.5% (w/v); MgSO₄·7H₂O, 0.02% (w/v); NaCl, 0.5% (w/v); biotin, 0.001% (w/v); K₂HPO₄, 0.25% (w/v) plus the carbon source, as indicated in each experiment. In other experiments, Lee's medium was used (Lee *et al.*, 1975) and cells were grown at 28 °C in the yeast form on this medium as reported by Brummel & Soll (1982).

Germ-tube formation. Yeast-form cells grown to stationary phase in any of the above media, were harvested, washed and resuspended to 10⁷ cells ml⁻¹ in one of the following media: basal saline (see above) with GlcNAc (4 mM) with or without glucose, or Lee's medium pH 6.5, as modified by Brummel & Soll (1982) but without leucine.

Cells were incubated at 37 °C, and germ-tube formation was evaluated by light microscopy for a period of 4–4.5 h. In all cases, although asynchronous, the time-course of morphogenesis was such that, starting from 0-time yeast cells, at 60–90 min there were germ-tubes already emerged and visible, and at 240–270 min elongated tubes with a septum, conventionally called hyphal cells, were formed. Yeast, germ-tube forming and mycelial cells will be often referred to in the text as different stages of yeast-mycelial transition.

Protein synthesis and turnover. Protein synthesis was measured by incorporation of radioactive amino acid by two experimental methods.

In the first, samples (5 or 10 ml) of cultures, growing at 28 or 37 °C, were withdrawn at intervals throughout the duration of the experiment and added to a temperature pre-equilibrated mixture containing the radioactive precursor in basal saline with or without supplementary nutrients, as indicated in individual experiments. At the end of each pulse (15 min), the incorporation was stopped by addition of 5 ml cold 20% (w/v) TCA containing 0.1% (w/v) unlabelled precursor. Samples were heated at 100 °C for 30 min to extract soluble material, cooled and the associated radioactivity determined as described below. In the second kind of experiment, cells at different stages of growth or germ-tube formation were harvested, thoroughly washed and reincubated in the basal medium containing the radioactive precursor, in the presence or absence of other compounds, as indicated. After 15 to 30 min, the incorporation was stopped and the samples were processed as described above.

Uptake of the radiolabelled amino acid into the soluble pool was determined at the desired time by extracting the pool by hot water, as described below and measuring the associated radioactivity.

Protein turnover was evaluated by incorporating the radioactive amino acid for 30 min in a 200 ml culture growing at 28 or 37 °C in the yeast form in a basal medium containing 0.5% (w/v) GlcNAc. The incorporation was stopped with cold, non-radioactive basal medium and the radioactivity incorporated into acid-insoluble material in a sample of the culture was measured (time 0). The culture was divided into 10 ml samples in 100 ml Erlenmeyer flasks and incubated at 28 or 37 °C. At different times, the residual radioactivity incorporated into acid-soluble

material was determined. In some experiments, trichodermin (20 μM) was added at different times to inhibit the incorporation, and in other experiments cells which had incorporated the amino acid precursor were heated for 30 min at 100 °C in 1 M-NaOH to extract the incorporated radioactivity (see Results).

Radioactivity determination. Radioactive cellular materials were collected on 0.45 μm membrane filters (type HA; Millipore). The precipitate was carefully washed three times with 5 ml 5% (w/v) TCA containing excess unlabelled amino acid, then with 5 ml cold ethanol (95%, v/v). Filters were dried in an oven, dissolved in Bray solution (Bray, 1960) and counted in a LKB-Wallac 1214 Rack beta liquid scintillation system. Counting efficiency was calculated by the two channels ratio by using the external standard method. To determine uptake of radiolabelled amino acid into the soluble pool, 1 ml extract (see above) was added to 6 ml Bray's solution and the radioactivity was measured.

Determination of dry weight. The organism in the different forms and phases of growth was harvested and filtered on pre-tared 0.8 μm membrane filters (Millipore). Filters were washed with 50 ml cold distilled water, dried in an oven at 80 °C for 12 h and weighed.

Amino acid pool extraction and determination. The amino acid pool(s) was extracted from cells at different stages of yeast-mycelial transition by hot water essentially as described by Sutherland & Wilkinson (1972). Briefly, 1-l cultures germinating in GlcNAc or Lee's medium were harvested at 0, 90 and 240 min, chilled in ice and filtered on 0.8 μm filters (Millipore). The filters were washed three times with cold distilled water, and the organism was collected in 10 ml distilled water and heated for 10 min at 100 °C. After centrifugation at 10000 *g* for 15 min, the supernatant fluid was recovered and lyophilized.

The lyophilized extracts were dissolved in lithium citrate buffer (0.15 M, pH 2.2) and proteins were precipitated in 10% (w/v) sulphosalicylic acid. After centrifugation each supernate was subjected to amino acid analysis in a Carlo Erba analyser (Milan, Italy), model 3A29, equipped with a Sigma 15 Perkin-Elmer recorder and a 3AR/6/DC/20 Resin Chromatographic Column under lithium form. Norleucine was used as internal standard and elution was carried out through a series of lithium citrate buffers.

Chemicals. *N*-Acetyl-D-glucosamine was purchased from Sigma and glucose was obtained from Merck.

Trichodermin was a kind gift of Dr D. Kerridge, Department of Biochemistry, University of Cambridge, UK.

The radiolabelled amino acids L-[2(n)-³H]methionine (specific activity 8.1 Ci mmol⁻¹; 299 GBq mmol⁻¹) and L-[U-¹⁴C]leucine (specific activity 330 mCi mol⁻¹; 12.2 GBq mol⁻¹) were purchased from Amersham.

RESULTS

Uptake and incorporation of radioactive amino acids during GlcNAc-induced morphogenesis

Previous results (Mattia *et al.*, 1982) showed that cellular protein content decreased significantly during morphogenesis induced by GlcNAc, equivalent to about 20% of cell dry weight in the 240 min mycelial cells as compared to the initial 32% of dry weight of yeast-form cells. Initially, the time-course of protein synthesis was followed during GlcNAc-induced morphogenesis by measuring the incorporation of the radiolabelled precursor given as a single, initial pulse. Mycelial-converting cells took up the amino acid precursor efficiently and incorporated it into TCA-insoluble material. The incorporation was blocked by the addition of 20 μM -trichodermin, a potent inhibitor of protein synthesis, which also inhibited hyphal conversion. The incorporated radioactivity was also fully extractable by hot 1 M-NaOH. Similar results were obtained using [¹⁴C]leucine or [³H]methionine as the labelled amino acids (data not shown). These results showed that there was effective protein synthesis during the initiation of hyphal conversion in GlcNAc and that this synthesis was essential for conversion to occur, as also shown by Shepherd *et al.* (1980*b*).

The preliminary experiments reported above, while showing the requirement of protein metabolism for induction of the mycelial form, could not detect differences in the rate or extent of protein synthesis in yeast, germ-tube forming or mycelial cells. To establish this point, two approaches were used: in the first, cultures were pulsed (for 15 min) at different times throughout the duration of the experiment (240 min), starting from 0 time, and protein synthesis was expressed as radioactivity incorporated into TCA-insoluble material by the culture at the time of addition of labelled precursor. In a second series of experiments, cells at different stages of yeast-mycelial transition (yeast, germ-tube forming and mycelial cells) were harvested, washed and incubated for 15–30 min in a mixture containing labelled amino acid.

When the rate of protein synthesis was measured after pulses given at various times during yeast-mycelial transition, two clearly distinct phases of incorporation were observed (Fig. 1).

Fig. 1. Radioactive pulse incorporation during yeast-mycelial transition induced by GlcNAc at 37 °C and expressed as radioactivity incorporated into hot TCA-insoluble material per 10^7 original cells (O) or per mg dry wt (●). The radioactive precursor [^3H]methionine (2.5 μCi) was added at the indicated time to 15 ml cultures (10^7 cells ml^{-1}) and the incorporated radioactivity was measured after 15 min. Values are the mean of two independent experiments with triplicate samples. Variation did not exceed 12%. For all other technical details see text.

The initial one, which morphologically corresponded to the phase which precedes the emergence of germ-tubes, lasted 30–60 min and was characterized by an increasing rate of incorporation. In the second phase, a progressive decrease in the incorporation was observed, so that the rate of protein synthesis by the culture at the stage of mycelial cells was always lower than that of original yeast-form cells and much lower than the maximum rate of synthesis recorded at the end of the first phase.

The extent of this decay, as well as the ratio between maximum and minimum incorporation at the end of the two phases, could differ quantitatively depending on the amount of radioactive precursor or other cultural conditions, but the two distinct phases were always observed. Similar results were obtained using strain 3153A instead of strain BP of *C. albicans*. Since cells undergoing the GlcNAc-induced hyphal conversion had a limited growth rate (40–50% increase in dry weight by 240 min; see also Mattia *et al.*, 1982), only slight quantitative variations were seen when the radioactivity was plotted against the initial number of yeast-form cells rather than against the dry weight (Fig. 1).

The progressive reduction in the ability of germ-tube forming and mycelial cells to incorporate labelled amino acid into proteins (as shown in the pulse experiments above) could reflect either a reduced biosynthetic capacity (including the uptake of the precursor into the soluble pool) or an augmented rate of protein catabolism. Data relevant to these questions came from experiments in which yeast cells, pre-labelled for 30 min at 28 or 37 °C with a radioactive amino acid, were grown in the yeast (28 °C) or hyphal form (37 °C) in GlcNAc medium. At different times during growth, the residual TCA-insoluble radioactivity was measured and its decay was taken as a crude indicator of protein catabolism. It was seen that labelled proteins were catabolized at a comparable rate by yeast-form growing cells and by hyphal-converting culture throughout the differentiation period. However, when uptake into the amino acid pool was evaluated, cells at any stage during yeast-mycelial transition had comparable rates of uptake (per mg dry weight). In particular, mycelial cells showed no marked decrease in the 15 min uptake of the radiolabelled precursor into the pool when compared with the yeast-form cells (Table 1).

Since GlcNAc is rapidly consumed during hyphal conversion (Shepherd *et al.*, 1980a), the decrease of protein synthesis described above could also be due to a partial deficiency of readily utilizable carbon/energy sources in the medium during the pulse of the incorporation of radio-

Table 1. *Leucine access to cellular water-soluble pool during yeast-mycelial transition in GlcNAc*

Cells at the indicated time during transition were given the radiolabelled amino acid. After 15 min, the incorporation was stopped in an ice-bath with excess cold leucine and samples were extracted for the soluble pool as described in Methods. The values are means of three independent experiments with triplicate samples. Variation did not exceed 15%.

Time (min)	Stage of induction	[¹⁴ C]Leucine incorporation*
0	Yeast	21.0
60	Yeast	19.2
90	Germ-tube	16.7
150	Germ-tube	19.0
240	Hyphal cell	17.4

* [¹⁴C]Leucine was used at a concentration of 0.174 μ Ci ml⁻¹ (specific activity 0.33 Ci mmol⁻¹; 12.2 GBq mmol⁻¹). Results are 10⁻³ $\times$ c.p.m. (mg dry wt)⁻¹.

Table 2. *Effect of the addition of amino acids (AA) and/or glucose (Glc) to the N-acetyl-D-glucosamine (GlcNAc) medium on protein synthesis in cells at different stages of yeast-mycelial transition*

GlcNAc was used at 4 mM and Glc at 50 mM. Amino acids were those present in Lee's medium (Lee *et al.*, 1975) without leucine. Results are 10⁻⁴ $\times$ c.p.m. (mg dry wt)⁻¹ and are means of three independent experiments with duplicate samples. Variation did not exceed 10%.

Stage of induction	[¹⁴ C]Leucine incorporation into TCA-insoluble material*		
	No addition	Plus AA	Plus AA and Glc
Yeast (0 min)	5.2	3.1	5.9
Germ-tube forming (90 min)	4.9	7.8	26.5
Mycelial (240 min)	2.4	10.2	33.7

* 15 min pulses at 37 °C with 0.2 μ Ci [¹⁴C]leucine per 5 ml culture (specific activity 330 mCi mmol⁻¹; 12.2 GBq mmol⁻¹) in the absence of amino acids and with 2 μ Ci in the presence of amino acids. For all other technical details see text.

active precursor. To answer this question, experiments were done where the effect of the addition of Glc or excess GlcNAc and/or amino acids was evaluated. The additions were made either at the time of addition of labelled amino acid (the nutrients were included in the medium containing the radioactive precursor, see Methods) or by including the nutrients in the GlcNAc-induction medium. The rate of protein synthesis under these conditions was compared to that occurring in synthetic glucose/amino acids medium (Lee's medium), which supported a high rate of protein synthesis, as shown by Brummel & Soll (1982). The addition of glucose or excess GlcNAc at the time of addition of labelled amino acid did not significantly modify the temporal changes associated with hyphal morphogenesis in GlcNAc, whereas the addition of amino acids reduced to about half the fall of incorporation during hyphal formation. Moreover, if the amino acids were present in the GlcNAc medium from the beginning of the induction, no fall in the protein synthesis was observed, but instead a gradual increase in incorporated label was evident, which was potentiated by the presence of glucose (Table 2). Overall, when hyphal transition was induced in the presence of GlcNAc plus Glc and amino acids, the rate of protein synthesis increased during transition in a way similar to that occurring in Lee's medium (i.e. with glucose and amino acids but without GlcNAc; Table 2).

Protein synthesis in cells separated at different stages of yeast-mycelial transition

The results of the pulse-incorporation experiments above did not exclude the possibility that protein synthesis could be quantitatively affected by changes occurring in the culture as it pro-

Table 3. *Effect of glucose (Glc) or N-acetyl-D-glucosamine (GlcNAc) on protein synthesis in separated and washed forms of growth of Candida albicans induced in GlcNAc/saline medium*

GlcNAc was used at 10 mM and Glc at 15 mM. Results are $10^{-5} \times \text{c.p.m. (mg dry wt)}^{-1}$ and are means of three independent experiments with duplicate samples. Variation did not exceed 10%.

Stage of induction	[^{14}C]Leucine incorporation into TCA-insoluble material*		
	Basal saline	Plus Glc	Plus GlcNAc
Yeast (0 min)	2.19	2.78	2.70
Germ-tube forming (90 min)	1.75	2.57	1.70
Mycelial (240 min)	0.50	1.27	0.64

* 15 min incorporation at 37 °C of 0.5 μCi [^{14}C]leucine. For all other technical details see text.

gressed to hyphal formation, and did not fully reflect different capacities of cells at different stages of the yeast-mycelial transition to incorporate radioactive precursor and synthesize proteins. For instance, during germ-tube formation in GlcNAc, the pH increased (from 4.5 to 5.5), GlcNAc itself was significantly consumed and catabolites accumulated in the medium. To investigate these aspects further, 0-time yeast cells, the 90 min germ-tube cells and the 240 min hyphal cells were separated, washed and reincubated for the desired period in the radiolabelled precursor mixture with or without added nutrients. This kind of experiment was also done with cells induced to hyphal conversion in modified Lee's medium (Brummel & Soll, 1982).

Separated yeast, germ-tube forming and mycelial cells had distinct protein biosynthetic capabilities, with mycelial cells showing the lowest values (Table 3). If glucose was added during the 15 min incorporation period, the rate of protein synthesis by the hyphal-forming cells, although significantly greater than the control (in basal medium alone), was in all cases lower than that of the yeast-form cells. Again, excess GlcNAc had no effect. Note that in these experiments, in contrast to the pulse-incorporation experiments reported above, even the early germ-tubes (60 min) showed significantly decreased incorporation of radioactivity when compared to yeast cells. These experiments also showed that washing and separation of the organism before the incorporation experiments were done could further decrease its biosynthetic capability (Table 3). This was also shown by the results from hyphal conversion in Lee's medium (which, as mentioned above, supported a high rate of protein synthesis in the pulse experiments). In fact, washed mycelial cells derived from Lee's medium also had a slightly decreased rate of incorporation of radioactive precursor in basal saline and only germ-tube forming cells showed a small increase in protein synthesis when compared with yeast cells (Fig. 2). The addition of excess glucose to the incorporation mixture was without effect. Only when washed germ-tubes and hyphae were assayed for incorporation in fresh Lee's medium was their protein biosynthetic capability unaltered or slightly increased with respect to that of yeast-form cells (Fig. 2).

Amino acid pools at different stages of yeast-mycelial transition in GlcNAc or Lee's medium

The results reported above, showing the importance of the availability of external amino acids for a sustained high rate of protein synthesis during the dimorphic transition, suggested a differing ability of yeast and mycelial cells differentiating in GlcNAc to synthesize amino acid from NH_4^+ and carbon sources which would, in turn, lead to differences in the size and composition of the amino acid precursor pool. It was important to evaluate these possible differences since they could greatly affect the efficiency of incorporation of radioactive precursor. This prompted us to determine the amino acid pools during yeast-mycelial transition. In the GlcNAc-induced dimorphic transition, the total amino acid concentration of the pool was 4.05, 2.83 and 3.96% of cell dry weight in yeast, germ-tube (90 min) and hyphal cells (240 min), respectively. There were significant changes in pool composition (Table 4).

Amino acids could be grouped broadly into three categories. Amino acids of the first group, mostly glutamine, glutamic acid and alanine, were similarly represented in the yeast and early

Fig. 2. Protein synthesis by washed forms of growth of *Candida albicans* separated from Lee's medium at the indicated times and incubated at 37 °C in the presence of [14 C]leucine (1 μ Ci) in basal saline (●), or basal saline plus glucose (50 mM) (△), or fresh Lee's medium without leucine (▲). After 15 min incorporation, the samples were processed as described in Methods. For all other details, see legend to Fig. 1 and text.

Table 4. *Amino acid pool composition in yeast, germ-tube and hyphal forms of Candida albicans differentiated in GlcNAc*

Results are given as percentage cell dry weight. Numbers in parentheses represent the $\pm$ percentage variation with respect to the amount in the yeast form. Values represent the mean of two independent experiments (differences in the range $\pm 15\%$) except those marked by †, which were from a single determination. The percentage increase/decrease was not calculated for those amino acids accounting for <0.1% dry weight in the yeast form. The yield of the water extracts was in the range of 20–22% cell dry weight in all forms of growth.

Group	Amino acid*	Yeast	Germ-tube	Hyphal
1	Glutamine	0.69	0.67 (–2.9)	1.34 (+93)
	Glutamic acid	0.98	1.03 (+5.6)	1.13 (+15)
	Alanine	0.18	0.24 (+37.1)	0.36 (+103)
	Glycine	0.02	0.05	0.06
	Half cystine	0.07	0.08	0.12
2	Aspartic acid	0.31	0.13 (–58)	0.16 (–48)
	Lysine	0.46	0.16 (–59)	0.23 (–50)
	Histidine	0.18	0.08 (–57)	0.08 (–57)
	Arginine	0.34	0.15 (–55)	0.15 (–55)
	Valine	0.25	0.03 (–88)	0.06 (–77)
	Leucine	0.12	0.02 (–83)	0.03 (–79)
	Tyrosine	0.22†	0.09† (–59)	0.12† (–45)
	Phenylalanine	0.04	0.01	0.01
3	Threonine	0.05	0.04	0.06
	Serine	0.10	0.07	0.09
	Proline	<0.01	<0.01	<0.01
	Methionine	0.01	0.012	0.012
	Isoleucine	0.10	0.018	0.062
	Total	4.05	2.83 (–30)	3.96 (–2.9)

* Citrulline, ornithine and cystathionine were also present in low amounts.

germ-tubes but increased relatively in the mycelial cells with glutamine and glutamic acid together accounting for 62% of total free amino acids in the hypha (against 41% in the yeast). A larger group of amino acids, including several basic and aromatic ones, showed a marked decrease both in germ-tubes and in hyphal cells. Lysine, aspartic acid and arginine, which were the predominant compounds of this category, showed an average reduction of about 60% after

90 min of induction and 50% after 240 min (mycelial cells). The decreased amount of this group of amino acids largely accounted for the total decrease in the pool at the germ-tube stage. A third group of amino acids including threonine, serine, proline, methionine and isoleucine did not show substantial variation during hyphal conversion. It should be noted that the total amino acid pool of mycelial cells was comparable quantitatively to that of yeast cells but this was due to the higher content of the first group of amino acids in mycelial cells. Therefore, during induction of the mycelial form in GlcNAc, there was a pronounced change in the relative proportion of several amino acids in the pool with respect to the original, 0-time yeast form. When similar determinations were made in cells undergoing mycelial conversion in Lee's mixture, germ-tube forming and mycelial cells had almost double the concentration of the total amino acid pool when compared with germ-tube forming and mycelial cells induced in GlcNAc-medium. There were also some variations in the qualitative pool composition among the different stages of hyphal induction but these variations were not comparable to those shown by GlcNAc-induced forms of growth.

DISCUSSION

In previous studies concerning protein synthesis during dimorphic transition in *Candida albicans* it has been found that protein synthesis is required for transition to occur (Shepherd *et al.*, 1980*b*) and that the rate of the synthesis and the total protein content are coupled with growth and morphogenesis (Brummel & Soll, 1982). The results reported in this study, while confirming the requirement of protein synthesis for yeast-mycelial conversion, show that hyphal morphogenesis may also proceed under progressive repression of protein synthesis, as evidenced by morphogenesis induction with *N*-acetyl-D-glucosamine (GlcNAc). The decay in protein synthesis appears temporally related to the hyphal-cell formation as it begins with initial germ-tube emergence (60–90 min) and progresses until the septate hyphal stage is reached (240–270 min). There were significant changes in the specific activity of several amino acids, including leucine, in the cellular pool during yeast-mycelial transition (see also below) but they could not explain the modulation in protein synthesis, as similar results were obtained when leucine was replaced by methionine, the concentration of which remained substantially unchanged in the pool. Moreover, the amino acid incorporation data are in accord with the diminution in total protein content during hyphal morphogenesis in GlcNAc previously reported by Mattia *et al.* (1982). If the rate of protein synthesis and total protein content are taken as indicators of cellular growth, the GlcNAc-induction system, in contrast to induction by Lee's medium (Brummel & Soll, 1982; Lee *et al.*, 1975) behaves as if there were an uncoupling between growth and morphogenesis. This contrasts with the observed slight linear increase in cell dry weight (approximately +20% and +50% of germ-tube and hyphal cell, respectively) during GlcNAc-induced morphogenesis. Note, however, that this increase in dry weight is not due to balanced cell growth but, substantially, to an augmentation of cell wall polysaccharides (Mattia *et al.*, 1982).

This sort of separation of morphogenesis from cellular growth appears as a characteristic aspect of GlcNAc-induced hyphal morphogenesis and adds interest to this model of eukaryotic differentiation.

The decay of protein synthesis which accompanies hyphal conversion in GlcNAc could depend on either a true diminution of biosynthetic ability (including changes in pool size and composition of precursors) and/or on a higher protein catabolic rate. Data obtained so far argue against hyphal cells catabolizing proteins at an exceedingly high rate as compared with yeast cells (unpublished observations). The rate of amino acid uptake into the cellular pool was also comparable in all forms of growth and, therefore, the hyphal-associated diminution of protein synthesis cannot be explained by a parallel degree of diminution in amino acid access to the cell interior. Nevertheless, the decay in protein synthesis was not due to the depletion of the carbon/energy source in the incorporation mixture since: (i) the rate of protein synthesis was not affected by the addition of an excess of GlcNAc under conditions of derepressed GlcNAc catabolism; (ii) the addition of glucose (up to 50 mM) to the incorporation mixture did not alter the incorporation values in GlcNAc. What appears to be important is the availability of external amino acids. This was shown by inducing the transition in GlcNAc plus amino acids (Table 2). In this case,

however, the presence of glucose seemed necessary for optimal utilization of amino acids for protein synthesis (Table 2; Brummel & Soll, 1982).

Variations in the endogenous synthesis of amino acids from NH_4^+ and GlcNAc may play a role in the regression of protein synthesis during hyphal conversion in GlcNAc. Although hyphal cells developed in GlcNAc do not appear to have limitations in glutamate and glutamine synthesis, they have a pool size about half that of hyphae developed in Lee's medium. They also show a significant diminution in the concentration of amino acids which require more enzymes and energy for their synthesis (Table 4). The results reported here, as well as the recognized importance of amino acid metabolism in the hyphal conversion in *C. albicans* (Dabrowa *et al.*, 1976; Land *et al.*, 1975), deserve further investigation.

The authors wish to thank Professor F. Bellomonte who helped in providing amino acid analyser facilities in the Laboratorio di Alimenti, Istituto Superiore di Sanità, Rome, Italy. This research was in part supported by grants from the Consiglio Nazionale delle Ricerche (Italy), contract n.83.00347, 96, Special Project 'Control of Infectious Diseases'.

REFERENCES

- AHRENS, J. C., DANEOMORE, L. & BUCKLEY, H. R. (1983). Differential protein synthesis in *Candida albicans* during blastospore formation at 24.5 °C and during germ tube formation at 37 °C. *Journal of General Microbiology* **129**, 1133–1139.
- BARTNICKI-GARCIA, S. & MCMORROUGH, I. (1971). Biochemistry of morphogenesis in yeasts. In *The Yeasts*, vol. II, pp. 141–191. Edited by A. H. Rose & J. S. Harrison. New York: Academic Press.
- BRAY, G. A. (1960). A simple, efficient liquid scintillator for counting aqueous solutions in a liquid scintillator counter. *Analytical Biochemistry* **1**, 279–285.
- BROWN, L. A. & CHAFFIN, W. L. (1981). Differential expression of cytoplasmic proteins during yeast bud and germ tube formation in *Candida albicans*. *Canadian Journal of Microbiology* **27**, 580–586.
- BRUMMEL, M. & SOLL, D. R. (1982). The temporal regulation of protein synthesis during synchronous bud or mycelium formation in the dimorphic yeast *Candida albicans*. *Developmental Biology* **89**, 211–224.
- DABROWA, N., TAXER, J. S. & HOWARD, D. H. (1976). Germination of *Candida albicans* induced by proline. *Journal of Bacteriology* **13**, 830–835.
- GOPAL, P., SULLIVAN, P. A. & SHEPHERD, M. G. (1982). Enzymes of *N*-acetyl-D-glucosamine metabolism during germ-tube formation in *Candida albicans*. *Journal of General Microbiology* **128**, 2319–2326.
- LAND, G. A., McDONALD, W. C., STJERNHOLM, R. L. & FRIEDMAN, L. (1975). Factors affecting filamentation in *Candida albicans*: relationship of the uptake and distribution of proline to morphogenesis. *Infection and Immunity* **11**, 1014–1023.
- LEE, K. L., BUCKLEY, H. R. & CAMPBELL, C. (1975). An amino acid liquid synthetic medium for development of mycelial and yeast forms of *Candida albicans*. *Sabouraudia* **13**, 148–153.
- LODDER, J. (1970). *The Yeasts: A Taxonomic Study*, pp. 914–978. Amsterdam: North Holland.
- MANNING, M. & MITCHELL, T. G. (1980). Morphogenesis of *Candida albicans* and cytoplasmic proteins associated with differences in morphology, strain or temperature. *Journal of Bacteriology* **144**, 258–273.
- MATTIA, E. & CASSONE, A. (1979). Inducibility of germ-tube formation in *Candida albicans* at different phases of yeast growth. *Journal of General Microbiology* **113**, 439–442.
- MATTIA, E., CARRUBA, G., ANGIOLELLA, L. & CASSONE, A. (1982). Induction of germ-tube formation by *N*-acetyl-D-glucosamine in *Candida albicans*: uptake of inducer and germinative response. *Journal of Bacteriology* **152**, 555–562.
- ODDS, F. C. (1979). *Candida and Candidosis*. Leicester: Leicester University Press.
- SHEPHERD, M. G. & SULLIVAN, P. A. (1983). *Candida albicans* germ-tube formation with immobilized GlcNAc. *FEMS Microbiology Letters* **17**, 167–170.
- SHEPHERD, M. G., GHAZALI, H. M. & SULLIVAN, P. A. (1980a). *N*-Acetyl-D-glucosamine kinase and germ-tube formation in *Candida albicans*. *Experimental Mycology* **4**, 147–159.
- SHEPHERD, M. G., CHEW, Y. Y., RAM, S. P. & SULLIVAN, P. A. (1980b). Germ tube induction in *Candida albicans*. *Canadian Journal of Microbiology* **26**, 21–26.
- SIMONETTI, N., STRIPPOLI, V. & CASSONE, A. (1974). Yeast-mycelial conversion induced by *N*-acetyl-D-glucosamine in *Candida albicans*. *Nature, London* **250**, 344–346.
- SINGH, B. & DATTA, A. (1979). Regulation of *N*-acetyl-D-glucosamine uptake in yeast. *Biochimica et biophysica acta* **632**, 345–353.
- SUTHERLAND, I. W. & WILKINSON, J. F. (1972). Chemical extraction methods of microbial cells. *Methods in Microbiology* **5B**, 349.