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Induction of Germ Tube Formation by *N*-Acetyl-D-Glucosamine in *Candida albicans*: Uptake of Inducer and Germinative Response

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A number of strains of *Candida albicans* were tested for germ tube formation after induction by *N*-acetyl-D-glucosamine (GlcNAc) and other simple (proline, glucose plus glutamine) or complex (serum) compounds. A proportion of strains (high responders) were induced to form germ tubes evolving to true hyphae by GlcNAc alone or by proline or glucose plus glutamine mixture. The majority of strains were low responders because they could be induced only by serum or GlcNAc-serum medium. Two strains were found to be nonresponders: they grew as pseudohyphae in serum. Despite minor quantitative differences, all strains efficiently utilized GlcNAc for growth under the yeast form at 28°C. They also had comparable active, inducible, and constitutive uptake systems for GlcNAc. During germ tube formation in GlcNAc, the inducible uptake system was modulated, as expected from induction and decay of GlcNAc kinase. Uranyl acetate, at a concentration of 0.01 mM, inhibited both GlcNAc uptake and germ tube formation and was reversed by phosphates. Germinating and nongerminating cells differed in the rapidity and extent of GlcNAc incorporation into acid-insoluble and alkali-acid-insoluble cell fractions. During germ tube formation induced by proline, GlcNAc was almost totally incorporated into the acid-insoluble fraction after 60 min. Moreover, hyphal development on induction by either GlcNAc or proline was characterized by an apparent "uncoupling" between protein and polysaccharide metabolism, the ratio between the two main cellular constituents falling from more than 1 to less than 0.5 after 270 min of development. The data suggest that utilization of the inducer for wall synthesis is a determinant of germ tube formation in *C. albicans* but that the nature and extent of inducer uptake is not a key event for this phenomenon to occur.

Germ tube formation from yeast-form cells of *Candida albicans* may be considered as a rapid, relatively simple model of cellular morphogenesis in fungi. One of the simplest methods to induce germ tube formation followed by extensive hyphal growth is to expose yeast-form cells to *N*-acetyl-D-glucosamine (GlcNAc), at 37°C (16, 18; E. Mattia, A. Benedetto, G. Carruba, and A. Cassone, Atti XIX Congresso Nazionale di Microbiologia, Catania, in press). It has been hypothesized (18) that the capability of GlcNAc to trigger hyphal development is somehow related to an activation of the synthesis of chitin (1), a minor but important cell wall polysaccharide, the content of which is markedly increased in germ tube (7) as well as in hyphal (5) cell walls.

Nevertheless, GlcNAc is readily utilized as a carbon energy source by many yeasts; in particular, it can be metabolized by *C. albicans* for growth in yeast or pseudomycelial forms. The key question to be answered then is, what biochemical event(s) in the metabolic pattern of GlcNAc may directly and specifically be related to germ tube formation? Along this line, the use of strains of *C. albicans* endowed with differences in germinative response to GlcNAc seemed a means to obtain insight into the mechanism(s) by which GlcNAc promotes germ tube formation.

This paper reports the results of such an approach and is primarily concerned with a comparison of different strains of *C. albicans* in growth, uptake of GlcNAc, and germ tube responsiveness.

MATERIALS AND METHODS

Organism and growth conditions. All strains of *C. albicans* used throughout this study were isolated from clinical specimens, except CA2, M1, and M3, which were selected as echinocandin-resistant mutants (3;

R. E. Mason, Ph.D. thesis, University of Cambridge, Cambridge, England, 1978) and kindly provided by D. Kerridge (Department of Biochemistry, University of Cambridge). The strains were identified by established taxonomic criteria (10). In particular, CA2, which is a non-germ tube-forming strain (see below) showed the sugar assimilation and fermentation patterns typical of *C. albicans* and was pathogenic for mice. All strains were routinely maintained in the yeast form on Winge solid medium at 28°C (12). For growth on single carbon sources, the microorganism was pregrown at 28°C in a peptone-phosphate liquid medium (peptone, 0.25% [wt/vol]; KH_2PO_4, 0.3% [wt/vol]; and biotin, 0.001% [wt/vol]) (19). This medium was supplemented with either glucose (Glc) or GlcNAc (5 g/liter). After reaching stationary phase, the organisms were diluted into fresh medium at 28°C to give an initial inoculum of 10^6 cells per ml. At different time intervals, growth was determined by direct counting in a Burke hemacytometer as well as by optical density measurements (1 cm; 560 nm). Unless otherwise stated, all experiments were performed with strain BP of *C. albicans*.

Germ tube formation. Yeast-form cells grown to the stationary phase were harvested from basal medium containing either Glc or GlcNac, washed, and suspended to a final density of 2.10^6 cells per ml in the following media: water-diluted horse serum (10% [vol/vol]; S.I.A.L., Rome, Italy), basal saline medium [$(NH_4)_2SO_4$, 0.5% (wt/vol); $MgSO_4 \cdot 7H_2O$, 0.02% (wt/vol); NaCl, 0.5% (wt/vol); biotin, 0.001% (wt/vol) plus GlcNAc (4 mM) or proline (Pro) (50 mM), and a mixture of Glc and glutamine (Gln) (both 2.5 mM). Cells were incubated at 37°C, and germ tube formation was evaluated under a light microscope for a period of at least 12 h. In some experiments, uranyl acetate (0.01 mM) was added to the germination mixtures.

Determination of GlcNAc uptake. The uptake of GlcNAc was determined under the following conditions: (i) Time-course uptake by nongrowing cells, preincubated with GlcNAc or not preincubated, to follow the uptake by the inducible or the constitutive systems, respectively (19). These experiments were made at both 28 and 37°C. (ii) Initial uptake rate (over a 2-min period) during growth in yeast or germ tube forms in a "high-responder" strain (see Results) after induction by GlcNAc or Pro.

For time-course uptake, stationary-phase yeast-form cells of the different strains of *C. albicans* grown in Winge liquid medium at 28°C were washed and suspended to a final concentration of 10^8 cells per ml in the basal saline medium with or without 20 mM GlcNAc at 28 or 37°C. After 3 h of incubation, cells were washed, suspended in a biotin-free basal saline medium containing appropriate amounts of [^{3}H]GlcNAc in a total volume of 0.2 ml (cell density, 5 × 10^8/ml) and incubated at 28 or 37°C. At different time intervals, the uptake of radiolabeled GlcNAc was stopped by adding 5 ml of 22 mM GlcNAc-containing cold basal medium, and cellular samples were counted for radioactivity incorporated into total cell material (see below).

To measure the initial rate of GlcNAc uptake during germ tube and hyphal formation, 50-ml cultures of stationary yeast-form cells of the BP strain (10^6 cells per ml) in the basal saline medium containing either GlcNAc (4 mM) or Pro (50 mM) were incubated at 37°C. At different time intervals, cultures were centrifuged, and the organism was suspended in basal medium, containing appropriate amounts of [^{3}H]GlcNAc, to a final concentration of 2 mg (dry weight) per ml and incubated at 37°C for 30 s to 2 min. Here again, the incorporation was stopped with 5 ml of GlcNAc-containing cold basal medium, and the radioactivity was measured.

Incorporation of [^{3}H]GlcNAc into cellular materials of *C. albicans* during germ tube formation. Yeast-form cells (10^6/ml, in a total volume of 50 ml), were suspended in basal saline medium containing either GlcNAc (4 mM) or Pro (50 mM) and given 50 μCi of [^{3}H]GlcNAc. The cultures were incubated at 37°C, and at different time intervals the incorporation was stopped with cold GlcNAc-containing basal medium. The amount of radioactivity incorporated into the acid-insoluble fraction was measured by suspending filtered cells in 0.5% $HClO_4$, heating at 80°C for 60 min to extract soluble material, filtering again, washing, and processing as for the radioactivity incorporated into total cell material. For the radioactivity incorporated into the alkali-acid-insoluble fraction, treatment with perchloric acid was followed by a 2-h treatment in 3% NaOH at 100°C, then by a 2-h treatment with 0.5 N CH_3COOH at 100°C, washing, and processing as above for radioactivity incorporated.

In pulse-chase experiments, 25-ml sample cultures (containing 5 × 10^6 cells per ml) were given 25 μCi of [^{3}H]GlcNAc in basal medium and incubated for 1 h at 28°C. The incorporation was stopped with 1 ml of cold GlcNAc (100 mM), and cells were immediately incubated at 28 or 37°C. At different time intervals during incubation, 5 ml of each culture was used for the determination of radioactivity incorporated into total cellular material, and the residual 21 ml was used for determination of radioactivity incorporated into acid-insoluble material, as described above.

Radioactivity determinations. Radioactive materials were collected on 0.45-μm membrane filters (type HA; Millipore Corp., Bedford, Mass.) and thoroughly

TABLE 1. Germ tube formation by different strains of *C. albicans* in different media[a]

Strain	Time (h)	% Germ tube formation in:			
		GlcNAc	Pro	Glc+Gln	Serum
BP	2	80	70	60	80
	4	90	90	90	90
	12	M+	M+	M+	M+
A72	4	90	80	50–60	90
	12	M+	±	±	M+
M1	4	0	0	0	30
	12	PM	PM	PM	50
CA2	4	0	0	0	0
	12	0, PM	0, PM	0, PM	0, PM

[a] GlcNAc, 4 mM; Pro, 50 mM; Glc+Gln, 2.5 mM each; serum, water-diluted horse serum (5% [vol/vol]); M+, complete submerged mycelial growth; PM, pseudomycelial growth; ±, mixed morphology. All compounds were dissolved in basal saline medium, adjusted to pH 7.2. For other details, see the text.

washed with water and ethanol. They were dried in an oven, dissolved in Bray solution (2), and counted in a Beckman LS 8I100 liquid scintillation system. Counting efficiency was calculated by the two-channels ratio by using the external standard method.

Determination of dry weight and protein and polysaccharide content. For dry weight determination, the organism was harvested and filtered on pretared 0.8-μm membrane filters (catalog no. AAWP0470 M, Millipore). Filters were washed with 50 ml of distilled water, dried in an oven at 80°C for 12 h, and weighed. The total protein content was determined by the method of Lowry et al. (11), and the polysaccharides were determined by the anthrone reagent as described by Herbert et al. (9). For determination of chitin content, cell walls were isolated, fractionated, and hydrolyzed by the procedure described by Chattaway et al. (5). The glucosamine present in the alkali-acid-insoluble fraction was estimated according to the method of Strominger et al. (21).

Chemicals. Glc, L-Pro, Gln, and biotin were obtained from Merck (Darmstadt, West Germany). Uranyl acetate and GlcNAc were purchased from Fluka (Buchs, Switzerland). Radiolabeled GlcNAc (*N*-acetyl-D-[1-^{3}H]glucosamine; specific activity, 11 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, United Kingdom).

All other reagents were of standard purity grade.

RESULTS

Comparative germ tube-forming ability of blastospores of several strains of *C. albicans* in different media. To characterize the induction by GlcNAc as compared with other inducers of germ tube formation, several strains of *C. albicans* were exposed to GlcNAc, Pro, Glc+Gln, or serum. More than 30 strains were tested; Table 1 gives four examples of the response obtained. Only some strains (about one-third) were capable of forming germ tubes in GlcNAc alone. These strains, called high responders, were also efficiently induced to form germ tubes by Pro or Glc+Gln. The great majority of all other strains were low responders in the sense that they formed germ tubes only in serum or GlcNAc-serum mixture. Only two strains (CA2 and A12) did not respond to any medium; both developed in the pseudomycelial form when suspended in serum at 37°C.

All high responders showed comparable kinetics of germ tube formation, with only slight, quantitative differences, depending on the inducer used (Table 1). Germ tube formation was usually complete after 4 h, and submerged mycelial development occurred between 8 and 12 h after the beginning of incubation (Table 1).

Utilization of GlcNAc for growth in the yeast form by strains of *C. albicans*. Since the capacity to form germ tubes in response to GlcNAc was apparently confined to a limited number of strains, we first wondered whether this simply depended on a kind of "nonspecific ability" of responder strains to merely utilize GlcNAc for growth. To assess this possibility, all strains were tested for growth on GlcNAc as the sole carbon source in a nitrogen basal medium, at both 28 and 37°C. Growth on GlcNAc was also compared with growth on Glc as a standard carbon energy source. Figure 1 shows the results obtained with strains BP, M1, and CA2 as representative of high responders, low responders, and nonresponders, respectively.

All strains were capable of effective utilization of GlcNAc as the sole carbon source for growth

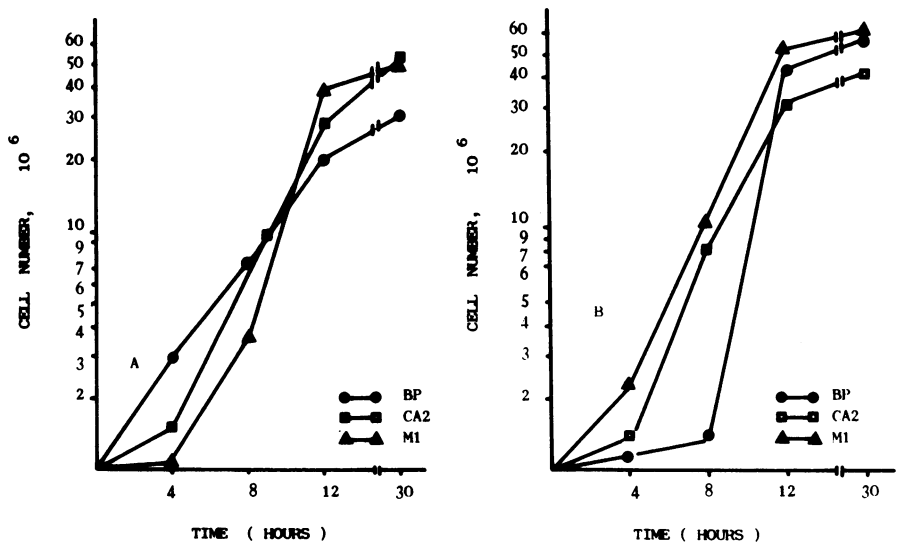

FIG. 1. Growth of different strains of *C. albicans* on GlcNAc (A) or Glc (B) in a peptone-phosphate medium at 28°C. For experimental details, see the text.

in the yeast form at 28°C. Moreover, the low responders and nonresponders also utilized GlcNAc for growth in the predominantly pseudomycelial form at 37°C, confirming the results shown in Table 1. On both Glc and GlcNAc, all strains ceased to grow after reaching a cell density of about 2×10^8 cells per ml (about 5 mg [dry weight] per ml) after 36 to 42 h of incubation.

GlcNAc uptake. Strains which were comparably efficient in GlcNAc utilization for growth but differed markedly in germinative response to GlcNAc were next examined for their mechanism of GlcNAc uptake both in resting cells and during germ tube formation (induced by either GlcNAc or Pro).

The results of our experiments confirmed previous reports by Singh and Datta (19) that GlcNAc is taken up by *C. albicans* by two distinct systems, one GlcNAc inducible and one constitutive (Fig. 2). This occurs in all strains examined, regardless of their GlcNAc responsiveness for germ tube formation. In particular, strains could be equally induced by GlcNAc to a high affinity uptake at both 28 and 37°C.

The uptake curves (Fig. 2) show that the overall kinetics of the inducible systems were not markedly different among all strains examined. Small differences were, however, noted in the initial uptake rate, and this reflected slightly different K_m values (not shown). The constitutive uptake system showed some differences among strains; in particular, the 30-min value of uptake in high-responder strains (BP and A72) was significantly lower than that of the low-responder and nonresponder strains (M1, M2, and CA2).

Values of K_m and V_{max} for the inducible and constitutive systems, expressed at 28 or 37°C, were calculated for strain BP and were similar to those previously reported by Singh and Datta (19).

Effect of uranyl acetate on germ tube formation and GlcNAc uptake. Uranyl acetate was shown

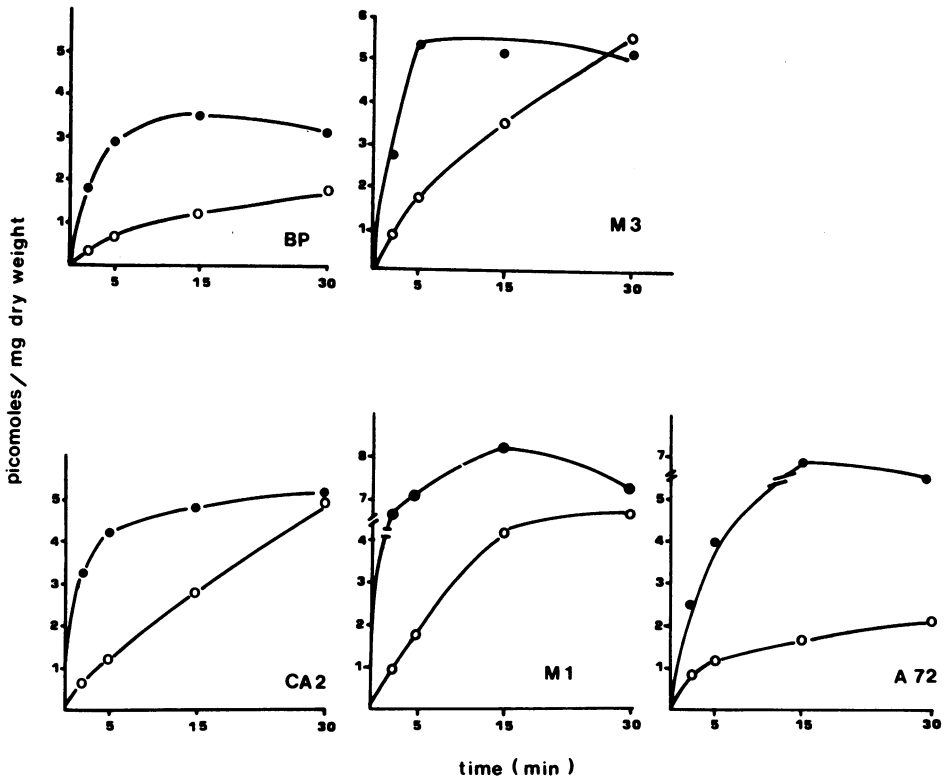

FIG. 2. Uptake of [^{3}H]GlcNAc by five different strains of *C. albicans* under resting conditions. Yeast-form cells were induced to the high-affinity uptake system (●) by preincubation with 20 mM GlcNAc for 3 h at 28°C. The constitutive uptake system (○) was evaluated in cells held as above in basal saline only. After incubation, cells were washed, adjusted to a density of 5×10^8/ml, and further incubated in prewarmed medium containing 0.5 μCi of [^{3}H]GlcNAc in a total volume of 0.2 ml.

TABLE 2. Effects of uranyl acetate on the uptake and germ tube formation by GlcNAc in two strain of *C. albicans*[a]

Strain	Uptake after 5 min (pmol/mg of dry wt)			Germ tube formation after 4 h at 37°C		
	−URA	+URA	% Inhibition	−URA	+URA	% Inhibition
BP, induced	9.0	0.05	99	>90	0	100
BP, uninduced	0.8	0.51	36.3	>90	0	100
CA2, uninduced	1.7	0.80	52.9	0	0	

[a] The experiments were performed as described in the legend to Fig. 2, except that uranyl acetate (URA) (0.01 mM) was added, and 2.0 μCi of [^{3}H]GlcNAc was used. The values are the means of triplicate samples.

to block the inducible transport system for GlcNAc without affecting the constitutive system (19).

In our experiments, this compound proved to be a powerful inhibitor of germ tube formation induced by GlcNAc (100% inhibition at 10 μM) (Table 2). It also inhibited the uptake of GlcNAc. This inhibition was complete for the inducible uptake system and partial (but significant) for the constitutive uptake system (36.3 and 52.9% inhibition for strains BP and CA2, respectively [Table 2]). The inhibition by uranyl acetate was fully reversible after withdrawal of the compound from the medium, nor did it occur when phosphate (Na^+ or K^+ salt, 30 to 40 μM) was present in the incubation mixture. Uranyl acetate was without effect on serum-induced germination, possibly because of serum phosphates.

TABLE 3. Initial uptake rate of GlcNAc at different time intervals during germ tube and hyphal development induced by GlcNAc or Pro

Time (h)	Uptake[a] of [^{3}H]GlcNAc during dimorphic transition in:			
	GlcNAc		Pro	
	pmol/min per mg of dry weight	pmol/min per mg of protein	pmol/min per mg of dry weight	pmol/min per mg of protein
0	0.90	2.65		
2	8.74	27.3	0.13	0.40
4	4.73	26.3	0.10	0.55
8	2.6	18.0	0.035	0.42

[a] The uptake was measured every 30 s in a 2-min interval of exposure of washed organism to [^{3}H]GlcNAc (10 μCi in 0.2 ml final volume) by calculating the radioactivity incorporated into total cell material. Over the indicated time interval, the rate was almost linear. The values are the means of triplicate samples. For all other technical details, see the text.

Uptake of GlcNAc during germ tube formation. Our second approach in the study of the relationship between GlcNAc uptake and germ tube formation was to examine possible modulations in the uptake system during germ tube formation. To avoid any interference by the germ tube inducer on the two distinct uptake systems, the constitutive uptake for GlcNAc was studied in cells induced to germinate by Pro (8). As reported above, there were no significant differences in the extent and kinetics of germination of high-responder strains in Pro or GlcNAc. Table 3 shows the rates of GlcNAc uptake by inducible and constitutive systems at different time intervals during germ tube formation. Marked modulation in the initial rate of GlcNAc uptake occurred during the dimorphic transition. The rate of inducible uptake increased during the first 2 to 3 h of incubation but significantly declined thereafter. This decline was much less pronounced when the rate of uptake was normalized to the total protein rather than the dry weight (Table 3). The rate of constitutive uptake of GlcNAc (germ tubes formed in Pro) also decreased but occurred later (i.e., during germ tube formation) and only when expressed on a dry weight basis.

Growth in the germ tube form and modulations in the protein and cell wall polysaccharide content in a high-responder strain. The data reported above suggested that changes in the ratio between protein content and total dry weight were likely to occur during germ tube formation and hyphal elongation in *C. albicans*. We measured this ratio and also determined the cell wall polysaccharide content at different time intervals during germ tube formation and hyphal elongation induced by GlcNAc. The results of such analytical determinations are summarized in Table 4 and Fig. 3. There is an apparent linear growth (as dry weight) to a 50% increase at 270 min. Over the same period of growth in the germ tube form, the protein content fluctuated, showing a minor increase (about 10%) by 150 min,

TABLE 4. Dry weight and total protein and polysaccharide content during germ tube formation[a]

Time (min)	Dry wt (mg)	Protein[b] (μg)	Polysaccharide[b] (μg)
0	1.00	370 (37)	301 (30.1)
30	1.08	378 (35)	347 (32.1)
90	1.20	391 (32.6)	404 (33.7)
150	1.32	401 (30.4)	479 (36.3)
270	1.52	274 (18.0)	599 (39.4)

[a] All values are normalized to 1 mg of dry weight of starting material (yeast-form cells).

[b] Values in parentheses represent the percentages relative to the dry weight.

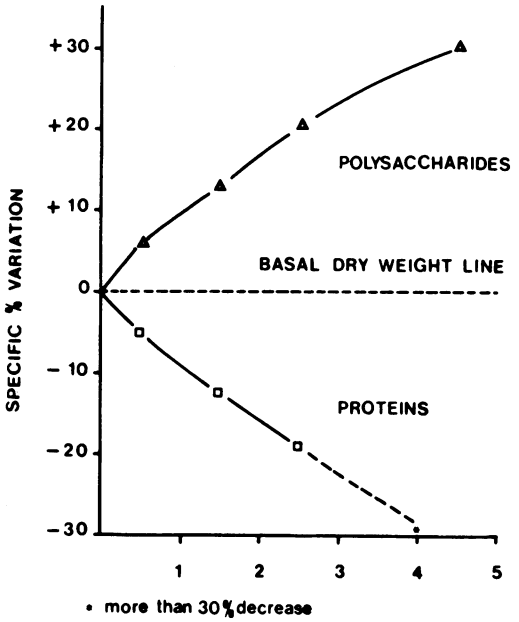

FIG. 3. Variation in total polysaccharide and protein content with respect to the dry weight during germ tube formation induced by GlcNAc in *C. albicans* BP. Abscissa: Time (hours).

followed by a marked decline in the subsequent 120 min. Conversely, total polysaccharide content rapidly and continuously increased, reaching 100% augmentation after 270 min. On a dry weight basis, therefore, there is an inverse relationship between protein, which decreased (from 37 to 18%) and polysaccharides, which increased (from 30 to almost 40%), during a 270-min period of GlcNAc-induced germ tube formation (Fig. 3).

In other experiments, we observed that among the principal cell wall polysaccharides, only chitin increased more than five times after 270 min. Mannan showed a little diminution late during mycelial development, whereas the ratio between glucan and total polysaccharides remained essentially constant until the 5th h of growth (Table 5).

TABLE 5. Changes in the relative content of cell wall polysaccharides during GlcNAc-induced mycelial conversion in *C. albicans*[a]

Time (min)	Chitin[b]	Mannan	Glucan[c]
0	1.6	25.0	73.3
30	5.9	23.8	70.2
90	6.6	23.0	70.3
150	7.5	19.0	73.4
270	8.4	18.7	72.8

[a] Values are the percentages of the total saccharides (chitin plus mannan plus glucan).

[b] Estimated as total hexosamine present in the alkali-acid-insoluble cell wall fraction (see text). The values are the means of triplicate experiments.

[c] Alkali-soluble plus alkali-insoluble glucan.

Incorporation of [^{3}H]GlcNAc during germ tube formation. The marked increase of polysaccharides relative to other cellular components (for instance, protein) appears as one of the most significant aspects of germ tube formation and suggests that high-responder strains are more prone, under these conditions, to use GlcNAc essentially for wall synthesis. Some support for this notion came from studies of [^{3}H]GlcNAc incorporation into cellular materials at early times of germ tube formation.

Table 6 gives the amount of [^{3}H]GlcNAc incorporated over a 60-min period into total cell, acid-insoluble, and alkali-acid-insoluble cell wall components in cells induced to form germ tubes by either GlcNAc or Pro. The data showed that as early as 30 min (still in the absence of any morphological sign of germ tube emergence), GlcNAc was preferentially incorporated into the acid-insoluble material. When Pro was used as inducer, the proportion of GlcNAc incorporated into the acid-insoluble fraction was very high, reaching almost 100% at 60 min.

A large fraction of [^{3}H]GlcNAc-associated radioactivity was found at 30 min in the alkali-acid-insoluble material which roughly corresponds to the cell wall ghost (alkali-acid-insoluble, glucan-chitin component), confirming that the inducer is metabolized early for cell wall biosynthesis, including its insoluble fraction. These results were confirmed in pulse-chase experiments performed with cells at 37 or 28°C (germinating or nongerminating, respectively) (Table 7). The amount of [^{3}H]GlcNAc incorporated into the acid-insoluble fraction at 37°C was significantly higher than that incorporated by

TABLE 6. [^{3}H]GlcNAc incorporation into different cellular fractions during germ tube formation[a]

Time (min)	Incorporation into total cell material (pmol/mg of dry wt)		% Incorporated into:		
			Acid insoluble		Alkali-acid insoluble
	GlcNAc	Pro	GlcNAc	Pro	GlcNAc
5	16.1	5.9	22.0	52.8	12.5
15	22.7		25.0		10.5
30	21.7	14.1	52.0	84.8	30.4
60	20.9	26.4	63.0	98.0	35.6

[a] [^{3}H]GlcNAc (50 μCi) was added at 0 time to a 50-ml culture of yeast-form cells (10^6 cells per ml) induced to form germ tubes by either GlcNAc (4 mM) or Pro (50 mM). At the indicated time intervals, the incorporation was stopped by GlcNAc-cold basal medium, and the radioactivity incorporated into distinct cellular fractions was measured as described in the text. The values are the means of duplicate experiments.

TABLE 7. Pulse-chase of [^{3}H]GlcNAc incorporation into the acid-insoluble fraction of *C. albicans* BP at 28°C (growth in yeast form) and 37°C (growth in germ tube form)[a]

Time (min)	[^{3}H]GlcNAc incorporated (pmol/mg of dry wt)		% Incorporation into total cell	
	28°C	37°C	28°C	37°C
0	2.4	2.4	6.6	6.6
15	3.2	4.41	18.4	30.0
30	2.8	6.50	18.5	44.3
60	5.6	6.6	31.0	50.0
120	10.6	23.7	55.7	84.0

[a] Stationary-phase yeast-form cells (5×10^6/ml) were incubated at 28°C in the presence of [^{3}H]GlcNAc (25 μCi) in a total volume of 25 ml. After 30 min, the incorporation was stopped by adding 1 ml of cold 100 mM GlcNAc, and the culture was incubated at 28 or 37°C. At the indicated time intervals, the radioactivity incorporated into the total cell and into the acid-insoluble fraction was evaluated as described in the text. The values are the means of duplicate experiments.

cells under nongerminating conditions. At 120 min, when all cells exhibited a mature germ tube, 34% of the GlcNAc was found in the acid-insoluble fraction.

DISCUSSION

Soon after the report (18) that GlcNAc, in a relatively low concentration and in the absence of any other carbon energy source, could efficiently trigger hyphal development in *C. albicans*, it was noted that strains of *C. albicans* may differ widely in response to GlcNAc (22; A. Cassone, unpublished data). Prompt and extensive germination in GlcNAc has been reported by Shepherd et al. (16) in cells "preconditioned" by starvation under aeration. We have shown here that under the usual conditions of germ tube formation assay (i.e., stationary-phase washed yeast-form cells, arising from a preculture in common medium), only certain strains (high responders) could be induced to germinate extensively and reproducibly in GlcNAc. It is of interest that only these strains also formed germ tubes when exposed to other simple compounds like Pro or Glc+Gln (8, 16). Dabrowa et al. (8) also noted variations in the germinative response to Pro by different laboratory strains and clinical isolates of *C. albicans*. All other strains (low responders) efficiently formed germ tubes only when more complex media, like serum or GlcNAc-serum mixtures, were used.

The "responsiveness" may be a set of physiological properties, and strains of *C. albicans* would therefore differ in the conditions under or the time by which these properties are manifested. The possibility also exists that germ tube formation is under strict genetic control, as suggested by occasional reports of "agerminative variants" of *C. albicans*. One of these "variants" seems to be the CA2 strain, primarily selected for its resistance to echinocandin (3; Mason, Ph.D. thesis).

Although the reasons for the different responses to simple germ tube inducers are not yet clear, our results seem to rule out the possibility that responsiveness depends on a selective or quantitatively different capacity to take up and metabolize GlcNAc. All strains, including the nonresponders, grew well on GlcNAc as the sole carbon energy source and were able to take up this compound by the inducible and constitutive uptake systems (17, 19). The slight differences of uptake among the strains were not apparently commensurate with the difference in the germinative response. On the other hand, Shepherd et al. (17) have shown that there is no relationship between the induction of GlcNAc kinase and germ tube formation. This is in keeping with our own results showing that GlcNAc uptake is comparably well induced (and expressed) in responder and nonresponder strains and at germ tube- and non-germ tube-competent temperatures (i.e., 37 and 28°C, respectively).

The modulation in the uptake of GlcNAc during GlcNAc-induced germ tube formation may be related to the time course of induction and the turnover of the GlcNAc kinase, as reported by others (17, 19). The increased rate of GlcNAc uptake during the first 2 h probably reflects the induction of GlcNAc kinase. Upon exhaustion of GlcNAc from the medium (which occurs after about 3 h), the kinase declines (17), and the uptake decreases to levels comparable to those of the constitutive uptake system.

If the mechanism of GlcNAc uptake bears no direct relationship to the germinative responsiveness, then this last property must rely on metabolic reactions allowing for increased wall synthesis during germ tube formation. We have shown here that during germ tube formation, GlcNAc is rapidly incorporated into acid-insoluble fractions which seemingly represent wall materials. In particular, when Pro was used as inducer (and, in a sense, GlcNAc was spared as a general carbon energy source), practically all GlcNAc taken up by the germinating cells was recovered in the acid-insoluble material. The net increase in the polysaccharide content during germination reflects the formation of new cell wall material, but more interesting seems the finding that, among mannan, glucan, and chitin, only the latter showed a specific increase relative to the total. This confirms previous findings and suggestions that chitin could play an important role in the mycelial conversion of *C. albicans* (5, 7, 18).

We have also shown here that a progressive "uncoupling" between protein and polysaccharides occurs to such an extent that the ratio between the two falls from more than 1 in the yeast form to less than 0.5 after 270 min of germination. Previous data on protein content in yeast-form cells, germ tubes, and hyphae of *C. albicans* are somewhat conflicting and cannot easily be compared with our own data due to the different conditions under and times by which germ tubes are formed in GlcNAc. However, the data reported by Chattaway et al. (4) show a trend toward a diminution of protein content in the hyphal forms with respect to a rather constant amount in the blastospores during the first 5 h of growth of the respective forms. In preliminary experiments, we observed that the rate of [^{3}H]methionine incorporation into acid-precipitable material markedly decreased during germ tube formation to a value of 50% that of blastospore after 80 min of incubation in GlcNAc at 37°C.

Recent results suggest that cAMP may be involved in germ tube formation in *C. albicans* (6, 14), analogous to what was reported in the dimorphic transition of *Histoplasma capsulatum* (13, 15). The elevated cAMP content found in germinating cells could be a factor in the fall of their protein content in view of the observation that cAMP inhibits protein synthesis in *C. albicans* (20). Clearly, the mutual relationship among cAMP, protein metabolism, and wall synthesis may be of relevance in the studies of GlcNAc germ tube formation in *C. albicans*.

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