The pathogenic fungus Cryptococcus neoformans has a polysaccharide capsule that is essential for virulence in vivo. Capsule size is known to increase during animal infection, and this phenomenon was recently associated with virulence. Although various conditions have been implicated in promoting capsule growth, including CO₂ concentration, osmolarity, and phenotypic switching, it is difficult to reproduce the capsule enlargement effect in the laboratory. In this study, we report that serum can induce capsule growth, and we describe the conditions that induce this effect, not only by serum but also by CO₂. Capsule enlargement was dependent on the medium used, and this determined whether the strain responded to serum or CO₂ efficiently. Serum was most effective in inducing capsule growth under nutrient-limited conditions. There was considerable variability between strains in their response to either serum or CO₂, with some strains requiring both stimuli. Sera from several animal sources were each highly efficient in inducing capsule growth. The cyclic AMP (cAMP) pathway and Ras1 were both necessary for serum-induced capsule growth. The lack of induction in the ras1 mutant was not complemented by exogenous cAMP, indicating that these pathways act in parallel. However, both cAMP and Ras1 were dispensable for inducing a partial capsule growth by CO₂, suggesting that multiple pathways participate in this process. The ability of serum to induce capsule growth suggests a mechanism for the capsular enlargement observed during animal infection.

The yeast Cryptococcus neoformans is a human pathogen that is ubiquitous in certain environments, such as soil contaminated with pigeon excreta. Human infection is believed to result from inhalation of infectious particles and, in some areas, 50 to 70% of individuals have antibodies against C. neoformans. However, cryptococcal disease in normal hosts is rare (22). Although most cases of human infection with C. neoformans are not recognized clinically, the infection can become latent and/or disseminate in the setting of immune impairment. The most common clinical manifestation of cryptococcosis is meningitis, a condition that is lethal unless treated.

C. neoformans has several virulence factors (for review, see reference 12), including a capsule, which is mainly composed of the polysaccharide glucuronoxylomannan (GXM). The size of the capsule of C. neoformans is variable, ranging from 5 to 30 μm (19, 43) and varying between strains (38). In soils and under laboratory conditions, the capsule size of most C. neoformans strains is relatively small but can increase during mammalian infection (5, 14, 33). Littman showed that the size of the capsule was highly variable and dependent on the environmental conditions (32). During in vivo infection, the size of the capsule varies depending on the organ studied. For instance, the lung environment is a powerful inducer of capsule growth (43). Another compartment that induces capsule growth, although not as efficiently as the lung, is the brain (18, 43). There are several reports indicating that the induction observed in vivo contributes to the virulence of the pathogen. Strains unable to induce capsule growth showed reduced virulence (3, 16, 23). In this regard, increase in capsule size has been associated with resistance to phagocytosis (8, 30, 37). But paradoxically, there is no correlation between capsule size at the moment of infection and virulence (3, 16, 24, 28). Several conditions induce capsule growth in vitro (for review, see reference 36). The most commonly used are high CO₂ concentration (23) and iron deprivation (25, 46). Other factors have also been described, such as availability of vitamins, the amino acids present, the type of carbon source (32) and osmolarity (17, 27). Unfortunately, it is difficult to achieve capsule growth under laboratory conditions, and not all the strains respond to these stimuli. Consequently, this phenomenon has not been extensively studied despite its relevance to cryptococcal infection.

The phenomenon of capsular growth is believed to be relevant because it increases the size of the cell and thus poses a problem for phagocytosis (29, 51). Furthermore, the capsule and the capsular polysaccharide interfere with a large number of processes involved in the immune response (15, 29, 34, 42, 47). The capsule is required for virulence, since acapsular mutants are avirulent (13, 21), and it is also necessary for survival inside phagocytic cells (45). However, several reports suggest that it is not necessary for protection in killing assays (9, 31).

Prior studies have shown that serum can have an inhibitory effect on yeast growth (41). However, studies about the effect of serum on capsule size are scarce (4), and it is not clear whether it can be used as a modulator of capsule growth. Here we demonstrate that sera from multiple sources can be potent inducers of capsule growth. We have also analyzed the requirement of CO₂ and gained insight into the putative pathways involved in this process. We conclude that both serum and CO₂ induce capsule growth and that this induction is also controlled by additional environmental factors.
**MATERIALS AND METHODS**

Strains and growth conditions. *C. neoformans* strains are listed in Table 1. Additionally, to study the role of cyclic AMP (cAMP) and Ras1 pathways, the following strains, kindly provided by J. Heitman, were used: RPC3 (cac1::URA5 [1]), RPC7 (cac1::URA5 CAC1 [3]), CDC16 (pka1::URA5 PKA1 [16]), CDC16 (pka1::URA5 PKA1 [16]), LCC1 (ras1::ADE2 [1]), and LCC2 (ras1::ADE2 RAS1 [1]).

Capsule growth induction. To study capsule growth, the yeast cells were incubated at 37°C for 3 hours. After incubation, the cells were washed and incubated with primary antibody 18B7 (10 μg/ml) at 37°C, washed, and incubated with GAM-immunoglobulin G-TRITC (5 μg/ml; Southern Biotechnology Associates, Inc., Birmingham, Ala.). After incubation at 37°C for 1 hour, the cells were suspended in mounting medium (50 mM n-propylgalactate, 50% glycerol in PBS) and observed with an Olympus AX70 microscope. To detect complement bound to the *C. neoformans* capsule, the yeast cells were grown in Sabouraud and washed with PBS, and 2 × 10^6 cells were suspended in 50 μl of mouse serum. After 1 hour of incubation at 37°C, the cells were washed and incubated with 24 h in PBS plus 10% FCS at 37°C.

**RESULTS**

Table 1. Induction of capsule growth by serum and CO₂

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serum PBS</th>
<th>No CO₂ Serum PBS</th>
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</thead>
<tbody>
<tr>
<td>H99</td>
<td>+</td>
<td>+/–</td>
</tr>
<tr>
<td>J4</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>J8</td>
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<td>J11</td>
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<td>J20</td>
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<td>J6</td>
<td>–</td>
<td>+/–</td>
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<td>J23</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>J24</td>
<td>+</td>
<td>–</td>
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<tr>
<td>102.97</td>
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<td>+/–</td>
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<td>+/–</td>
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<tr>
<td>16</td>
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</table>

* Yeast were grown in Sabouraud medium and transferred to PBS in the presence or absence of 10% FCS and in the presence or absence of 10% CO₂. Capsule size was determined after examining the cells in an India ink suspension. –, no induction; +/–, induction of 1 to 2 times; +, induction of >2 times.

Immunofluorescence. To detect the capsule of *C. neoformans*, a monoclonal antibody to GXM 18B7 (11) and a goat anti-mouse (GAM) immunoglobulin G conjugated to tetramethylrhodamine isothiocyanate (TRITC) were used. Briefly, cells were incubated in 1% bovine serum albumin-0.5% horse serum for 1 hour at 37°C, washed, and incubated with primary antibody 18B7 (10 μg/ml) for 30 minutes at 37°C, washed, and incubated with GAM-immunoglobulin G-TRITC (5 μg/ml; Southern Biotechnology Associates, Inc., Birmingham, Ala.). After incubation at 37°C for 1 hour and a final wash, the cells were suspended in mounting medium (50 mM n-propylgalactate, 50% glycerol in PBS) and observed with an Olympus AX70 microscope. To detect complement bound to the *C. neoformans* capsule, the yeast cells were grown in Sabouraud and washed with PBS, and 2 × 10^6 cells were suspended in 50 μl of mouse serum. After 1 hour of incubation at 37°C, the cells were washed and incubated with 24 h in PBS plus 10% FCS at 37°C.

Statistics. The data were assessed for normal distribution by using the Shapiro-Wilk test. For measurements where the data were normally distributed, statistical analysis was done with an analysis of variance and t test. For measurements where the data were not normally distributed, statistical analysis was done by using the Kruskal-Wallis statistic. P values of <0.05 were considered significant. All the statistics were performed with the Unistat 5.5 (Unistat Ltd., London, England) and Analyze-it (Analyze-it Ltd., Leeds, England) software for Excel.
phenomenon systematically, we studied the effect of both serum and CO₂ in the induction of capsule growth. We examined capsule growth of strain H99, because this strain was used in prior capsule studies (19, 23, 40) and most of the auxotrophic mutants are derived from this strain (39, 48). Under laboratory growth conditions, the capsule of H99 has a relatively small size, which is in marked contrast to the large capsule variants observed during murine infection (19). We first studied the capsule induction in the presence and/or absence of CO₂ and/or 10% heat-inactivated FCS. Capsule induction was studied in three different media: PBS, Sabouraud, and DME. As shown in Fig. 1A, in the absence of CO₂ serum induced a prominent capsule in strain H99 when the cells were incubated in PBS, indicating that serum alone is a potent inducing factor for capsule growth. However, when H99 was cultured in Sabouraud or DME, serum did not induce capsule growth. We repeated this experiment, but in an atmosphere containing 10% CO₂. In DME, capsule growth occurred in response to CO₂, even in the absence of serum. This result indicated that the growth medium affected the ability to induce capsule growth and suggested the need for additional stimuli. Most of the experiments described in this paper were carried out in PBS because its composition is defined, and in this solution the effect of serum on capsule growth was prominent. Serum-induced capsule growth at concentrations of 5% or higher, whereas below 5% the proportion of cells exhibiting increased capsule size was very small. Hence, we selected a serum concentration of 10% as our standard, because at this concentration strain H99 consistently demonstrated induced capsule growth. The induction of capsule growth was noticeable after only 6 h of incubation in inducing medium. Full induction of capsule growth, however, required 24 h (results not shown). The kinetics of capsule growth were not affected by incubation in CO₂.

Sera from fetal calf, mouse, human, rat, and guinea pig induced capsule growth (Fig. 2). There were no significant differences in the capsule volume of cells incubated in sera from different animals; however, there were small differences between mammalian sera, with rat and human sera being the most and less effective, respectively. Heat inactivation of serum had no effect on capsule induction (results not shown), suggesting no role of complement in the induction process.

Effect of pH and temperature on the serum-induced capsule growth. Several reports have indicated that capsule induction is a pH-dependent phenomenon (18, 23, 44). We observed that in the media in which the size of capsule increased, the pH was higher than 7. This suggested that a pH higher than 7 was necessary to induce capsule growth. However, pH was not a sufficient stimulus to induce capsule growth, since in a medium such as DME (pH around 7.5), serum did not induce capsule growth in the absence of CO₂. To investigate the effect of pH, we adjusted the pH to 5.6, from the slightly alkaline value of 7.3 found under our induction conditions (PBS plus serum). However, at a pH of 5.6, the serum did not efficiently increase capsule size, with only 15% of the cells having a large capsule, which was defined as a diameter of more than 45% of the total volume of the cell, capsule included (Fig. 3A).

Given the inefficiency of capsule induction by serum at pH 5.6, we investigated whether the lack of capsule growth in serum-supplemented Sabouraud medium was due to the acidic pH of this medium (range, 5.5 to 6). Consequently, we adjusted the pH of Sabouraud medium to 7.5 and studied serum induction. After incubation with the yeast, the pH of the suspension dropped to 7, but only a small proportion of cells (around 15%) demonstrated an increase in capsule size. However, statistical analysis indicated that the proportion of capsule in the cells after overnight incubation in Sabouraud without agitation in the presence or absence of serum decreased slightly (Fig. 3B). This indicates that in Sabouraud medium the lack of capsule induction was not due to the low pH of the medium.

The effect of temperature on capsule growth was studied. As shown in Fig. 4A, serum induced capsule growth at 24, 30, and 37°C, but the volume of the capsule was significantly larger at 37°C. However, the differences between the absolute volumes did not correlate with differences in the relative amount of capsule compared to the size of the cell. As shown in Fig. 4B, the percentage of the capsule after induction was very similar at all temperatures. To investigate the discrepancy between the data referred in volume or in percentage, we correlated the volume and the diameter with the size of the cell and found
positive correlations between both parameters (Fig. 4D). The apparent discrepancy arises because the size of the cell body is smaller at lower temperatures (Fig. 4C). So, we conclude that temperature does not have any effect on the proportion of capsule produced by the cell, but it does affect the size of the cell body.

Capsule growth in the presence of serum and CO₂ is highly strain dependent. Prior studies have noted differences in capsule growth among strains (19, 23, 32). To investigate this possibility, we compared capsule induction among various strains, including 24067 (serotype D) and H99 (serotype A). Strain 24067 exhibited two major differences compared to H99.
First, it manifested capsule growth in PBS in the presence of CO₂, without serum and additional nutrients (Table 1). Second, induction of capsule growth in strain 24067 was accompanied by a great heterogeneity in cell size and shape, such that cells with big capsules were mixed with other cells with a very small size of both cell body and capsule. In contrast, capsule induction in H99 produced a more homogenous population, with more than 95% of the cells demonstrating large capsules. We found that the cells that were placed in the induction medium had increased capsule size, and the small cells with small capsules were buds originated during the overnight incubation. We confirmed this finding by labeling the cells at the beginning of the incubation with C, which binds covalently to the capsule and does not segregate to the daughter cells. After overnight incubation in serum, none of the cells with a small capsule had C labeling, whereas more than 90% of the cells with a large capsule had C bound to the capsule (results not shown).

Capsule induction by serum and CO₂ was studied in other serotype A, B, C, and D C. neoformans strains. We found great variability in the response of different strains to the stimuli for capsule induction (Table 1). Serum strongly induced capsule growth in most serotype A strains, whereas for strains of this serotype CO₂ had little or no effect. In contrast, most of the serotype B strains responded very efficiently to either CO₂ or serum, and the combination of both stimuli induced a strong increase in capsule size for five of six strains. However, the serotype C strains studied did not respond to serum but did manifest increased capsule growth when exposed to CO₂. Serotype D strains demonstrated considerable variability in response to both serum and CO₂. For two strains (24067 and 13), both serum and CO₂ efficiently induced the capsule, whereas this induction was absent in other serotype D strains. We considered the possibility that the inability of serum to induce capsule growth for some strains was due to a limiting concentration of serum. Hence, we repeated the experiments using 100% FCS with seven different strains that did not respond to serum, but we did not observe induction of capsule growth.
serum- and CO₂-induced capsule growth.

We investigated whether capsular polysaccharide was involved in induction of capsule growth by studying the responsiveness of mutant strains lacking the adenylate cyclase (CAC1) and the cAMP-dependent protein kinase (PKA1) to serum and CO₂. When cells from these mutant strains were incubated in 10% FCS containing different amounts of ferric-EDTA in the absence or presence of 10% CO₂, Controls without iron and without serum were also studied. The average and standard deviation of capsule volume (A) and relative size of the capsule (B) under various conditions where FCS was supplemented with Fe are represented. Open bars denote the data of cells incubated in the absence of CO₂, and closed bars denote that in cells incubated in 10% CO₂.

Serum-induced capsule growth is not due to iron limitation. Iron limitation can stimulate capsule growth (46). Since serum contains iron-binding proteins that sequester iron, we explored whether the phenomenon of serum-induced capsule growth was due to iron limitation. Supplementation of serum-containing medium with different concentrations of iron did not inhibit the induction of capsule growth regardless of the presence or absence of CO₂ (Fig. 5).

The cAMP pathway and RAS1 are involved differently in the serum- and CO₂-induced capsule growth. cAMP is necessary to increase capsule size under conditions of iron limitation (3, 16). Hence, we investigated whether this pathway was involved in induction of capsule growth by studying the responsiveness of mutant strains lacking the adenylate cyclase (CAC1) and the cAMP-dependent protein kinase (PKA1) to serum and CO₂. When cells from these mutant strains were incubated in 10% FCS, no capsule growth was observed (Fig. 6). Since induction of capsule growth by serum was not observed in these mutant strains, we investigated whether capsular polysaccharide was present by indirect immunofluorescence using a monoclonal antibody to GXM. All the mutants were positive for the staining, indicating the presence of a capsule. Hence, absence of capsule induction was not a consequence of a lack of an encapsulated phenotype. Measurement of capsule volume and relative size of mutant strains under conditions of capsule growth induction revealed no significant differences relative to cells in the control media (Fig. 7A and B). In contrast, when CO₂ was used as a stimulus for capsule growth, both cac1 and pkal cAMP mutants manifested capsule growth compared to control condition, although the induction was significantly lower than that observed in the reconstituted or wild-type strains (Fig. 6 and 7C and D).

We also studied the role of Ras1 in the serum- and CO₂-induced capsule growth. RAS1 encodes a G-protein which has been mainly involved in C. neoformans in the control of the mitogen-activated protein kinase pathway (49). In the presence of serum, ras1 mutant cells failed to induce capsule growth (Fig. 6 and 7A and B). These experiments were performed at 37°C, a temperature where ras1 mutants show impaired growth (1). So, we repeated this experiment at room temperature and 30°C, but ras1 mutant cells did not manifest capsule growth at the lower temperatures. When CO₂ was used as inducing factor, ras1 mutant cells induced significant capsule growth (Fig. 6), although the size was smaller than the size of the complement strain (Fig. 7C and D).

We considered that the lack of serum-induced capsule growth for the ras1 mutants could reflect a defective cAMP pathway, which would involve an activation of the adenylate cyclase by Ras1. Hence, we studied the capsule induction by serum of ras1 mutant cells in the presence of exogenous cAMP. Addition of cAMP increased capsule size in the wild-type strain compared to the control without cAMP, but it did not have any effect on the behavior of the ras1 mutant (results not shown), suggesting that a defective cAMP pathway was not the cause of the lack of capsule induction by serum in the ras1 mutant.

DISCUSSION

Capsule growth is a morphological response of C. neoformans to a variety of stimuli, including infection of mammalian hosts. Here we report that incubation of C. neoformans in serum induces an increase in the capsule volume. Furthermore, we have studied the relationship between this phenomenon and other stimuli that are known to induce capsule growth, such as iron and CO₂ levels. To our knowledge, the serum induction phenomenon was unknown in the cryptococcal field. Previous studies have used a serum-containing medium to induce the capsule size (6, 7), but neither demonstrated that the effect was due to serum. In fact, both studies employed conditions that included other factors that can increase capsule size, such as a 5% CO₂ atmosphere. Other studies (4, 32) reported no induction of capsule growth when using human pooled serum, although one of these reports (4) described capsule induction by lyophilized rabbit coagulase plasma.

One striking finding of the serum inducing effect was its dependence on the composition of the medium. When cells were incubated in PBS with serum, capsule growth was induced, whereas in Sabouraud medium no increase in capsule
FIG. 6. Role of the cAMP pathway and Ras1 on serum- and CO2-induced capsule growth. (A) The strains H99, RPC3 (cac1), RPC7 (cac1 CAC1), CDC1 (pka1), CDC16 (pka1 PKA1), LCC1 (ras1), and LCC2 (ras1 RAS1) were incubated in the presence of 10% CO2 in DME medium or in PBS containing 10% heat-inactivated FCS in the absence of CO2. The pictures show cells in a suspension of India ink after incubation for 24 h, and the scale bar in the first picture (10 μm) applies for the rest of the pictures in the rest of the column.
size was observed. Dykstra et al. (17) reported that a high concentration of glucose (16%) repressed capsule induction, and they correlated this phenomenon to changes in osmolarity. However, Littman observed that the capsule induction by thiamine was not prevented by the addition of 10% glucose (32). We do not think that this explanation is relevant to our observations, since the glucose concentration under our conditions was lower (2%). With regards to induction by CO2, Granger et al. (23) demonstrated that capsule induction by CO2 only occurred in DME with 22 mM NaHCO3. Although we did not find that the addition of NaHCO3 to the DME was required for capsule growth, this discrepancy could be due to the different CO2 concentrations used in each study. Furthermore, it is conceivable that the requirement for NaHCO3 is not applicable to all C. neoformans strains. The dependence of the phenomenon on the composition of inducing medium may explain why the serum-induced capsule growth has not been reported before, despite the fact that C. neoformans is commonly incubated in solutions containing serum during immunological studies.

Another factor that influences capsule growth is the pH of the medium. Solutions with a pH lower than 7 inhibited the induction of capsule growth. This is consistent with the fact that all the media reportedly used to induce capsule growth (such as low-iron medium) have a pH around 7.3. The importance of the pH has been previously noted (18, 23, 44), and it is known that a basic pH can enhance capsule growth (17) and affect the morphology of the colonies on plates. Although a pH higher than 7 is required to induce capsule growth by serum, it is not a sufficient condition, since increasing the pH in media that do not allow capsule growth in the presence of serum, such as Sabouraud, did not result in larger capsules. The mechanism by which pH regulates capsule growth is not known, but in other organisms, such as C. albicans, morphological transitions are pH dependent (10). Sera from each of the four different mammalian species tested induced capsule growth. This result suggests the existence of a common inducing factor in mammalian sera. We noted some differences in the efficiency of the capsule growth induction, which could be related to differences in the concentration of the inducing compound. Littman studied the assimilation of most of the cerebrospinal fluid components by C. neoformans and their effect on capsule size (32). He reported that the most common lipids found in nervous tissue did not affect capsule size in vitro, whereas glutamic acid did induce capsule size. One of the most abundant proteins in serum is albumin, and we studied its role on capsule growth but did not find any effect of this protein (results not shown). Iron deprivation is one of the main factors that induces capsule growth (46). In our study, iron deprivation did not explain the capsule induction, since addition of saturating concentrations of iron did not prevent the induction. The identification of the

![FIG. 7. Capsule size after serum- and CO2-induced capsule growth in mutant strains of the cAMP and Ras1 pathways. Histograms in panels show capsule sizes of the mutant cells described in the legend for Fig. 6. The bars denote the mean and standard deviation of at least 20 cells after growth in Sabouraud (open bars), after 24 h of incubation (closed bars) in PBS plus 10% heat-inactivated FCS (A and B), or in DME and 10% CO2 (C and D). Determined as described in Materials and Methods, results in panels A and C represent the absolute capsule volume and results in panels B and D represent the relative size of the capsule. Asterisks denote statistical differences (*P < 0.05) in capsule size between time zero and 24 h of induction.](http://iai.asm.org/content/10/11/6162/F7.large.jpg)
inducing compound present in the serum is an important future goal that is outside the scope of this study.

While evaluating the role of temperature in the capsule growth process, we noticed a strong correlation between capsule size and cell size. Although at 37°C there was a larger capsule volume, the percentage of volume corresponding to the capsule was the same in all the cases. This result is in agreement with previous work that indicated that a shift from 24 to 37°C did not affect the proportion of capsule present in the cells (25). This suggests that in some strains the cell regulates the size of the capsule after induction. This is potentially a very interesting finding, because it implies the existence of mechanisms to control capsule size and thus avoid unlimited growth of the capsule. With regards to cell size, a potential control mechanism is cell cycle. In other yeast (50), the size of the cell determines the moment of cell division, and it is conceivable that in *C. neoformans* the process of capsule growth induction by serum is regulated not only by the cell size but also by the capsule size.

We observed considerable interstrain variability with regards to the response to serum. In general, serotype A and B strains manifested fewer interstrain differences. However, serotype D strains were highly variable in their response to serum. For some serotype D strains, such as 24067, we observed the simultaneous presence of macro- and microforms in the inducing medium. Similar microforms and heterogeneity have been described in vivo (19). Under our conditions, this heterogeneity was probably caused by the absence of capsule induction by the daughter cells arising during the incubation in serum. We do not have an explanation for this phenomenon, but it could represent phenotypic switching or changes in the medium during the incubation of the yeast, which would make this medium no longer efficient in inducing capsule size for the new cells produced. For other strains, such as H99, the presence of serum induced a homogenous response, although it has been reported that H99 can undergo a great variability after long incubations under conditions of capsule growth (23). The interstrain differences indicate the importance of the genotype for the capsule growth response. Our results establish great interstrain variation in capsule growth in response to serum, CO₂, and the inducing medium components. Consequently, the optimal conditions for each strain must be determined empirically.

cAMP is involved in *C. neoformans* capsule growth under low-iron conditions (2, 3, 16). Hence, we studied whether this pathway was required for induction of capsule growth by serum or CO₂. The cAMP pathway and Ras1 were each essential for induction of capsule growth by serum. Since Ras1 seems to act mainly through a cAMP-independent pathway (mitogen-activated protein kinase pathway [1]), we interpret this result as implying that serum induction is required for the interplay of several independent pathways. However, although reduced, some degree of capsule growth was found in the presence of CO₂ in the cAMP and ras1 mutants. Hence, RAS1 seemed to play a role under these conditions, even though ras1 mutants have impaired growth at 37°C (1). It is possible that the putative role of Ras1 is performed under these conditions by the homolog Ras2 (48). On the basis of the observations with the various mutants, we conclude that CO₂-induced capsule growth can occur through different pathways. At this point, we cannot distinguish whether both cAMP and Ras1 pathways cooperate as different but overlapping pathways or if there is another different pathway involved in the induction. Interestingly, induction of capsule growth by a low iron concentration seems to involve only the activation of the cAMP pathway, without any involvement of Ras1 (3, 16). This indicates that the growth of the capsule responds to different stimuli that are integrated by different pathways of the cell which, according to the conditions, will act in an overlapping manner or, in some other way, cooperate to increase capsule size efficiently.

Our results establish that capsule growth is induced in *C. neoformans* strains by mammalian sera. This effect may contribute to virulence by promoting the induction of large capsule variants after animal infection. Depending on the *C. neoformans* strain, this effect is independent of, or can be enhanced by, CO₂. Serum-mediated growth of the *C. neoformans* capsule provides a potential explanation for the observation that cells in tissue often manifest large capsules that are not evident when grown in fungal media in vitro.

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REFERENCES


