Role of Amino Acids and Endogenous Protein in the Germination of *Mucor racemosus* Sporangiospores

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The role of amino acids as triggers of *Mucor racemosus* sporangiospore germination was investigated. No single amino acid was as effective as glucose or peptone at triggering germination. Germination induced by glucose or peptone was pH-independent, whereas germination induced by glutamate was pH-dependent. The composition of the free amino acid pools of M-spores (those unable to germinate on glutamate) was qualitatively and quantitatively similar to that of C-spores (those capable of germinating on glutamate) with the exceptions of hydroxyproline and methionine whose concentrations were several-fold higher in C-spores. Glutamate and leucine were taken up by germinating and non-germinating spores; however, significant protein synthesis occurred only in germinating spores. Spores not triggered by 3-*O*-methyl-D-glucose (M-spores) contained about one-half the protein of those triggered by 3-*O*-methyl-D-glucose (C-spores). C-spores initiated to germinate by 3-*O*-methyl-D-glucose decreased in total organic carbon and protein over a 6 h period; removal of the 3-*O*-methyl-D-glucose resulted in an immediate halt of protein degradation and spore swelling. These results suggest that protein is a major endogenous reserve in *M. racemosus* sporangiospores and that its turnover is a necessary event for glucose-triggered germination.

INTRODUCTION

Sporangiospores of *Mucor racemosus* treated with a germinant undergo a morphological transition from an elliptical spore of 20 μm^3 to a spherical cell of 130 μm^3 with subsequent germ tube formation (Tripp & Paznokas, 1981). Analysis of the germination synchrony led to the observation that two physiologically distinct spore types exist. Sporangiospores produced on a nutritionally complex medium, denoted C-spores, are induced to germinate by a wide variety of compounds including glucose, cellobiose, xylose or glutamate. They are also triggered to initiate germination and increase in volume to about 90 μm^3 by several non-metabolizable glucose analogues such as 3-*O*-methyl-D-glucose (3-OMDG) (Tripp & Paznokas, 1982). The initiation signal was shown to be required continuously since the removal of 3-OMDG resulted in an immediate halt in swelling; re-addition of 3-OMDG restored swelling. Sporangiospores produced on a defined minimal medium, denoted M-spores, are triggered by only a few specific compounds structurally resembling glucose, or by nutritionally complex mixtures such as peptone. Non-metabolizable glucose analogues also trigger M-spores, but only in the presence of an exogenous carbon source such as glutamate. M-spores can be made competent to germinate on glutamate if they are pretreated with glucose for 90 min. Protein synthesis was necessary for the development of competence.

In the present study, we have investigated the relationship between sporangiospore germination ability and the intracellular content of various components such as amino acids,

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organic carbon and protein. Uptake and incorporation of glutamate and leucine during germination was also examined. It is suggested that glucose may trigger germination by signalling the breakdown of endogenous protein reserves. The subsequent increase of free amino acids may be the dormancy-breaking step.

METHODS

Organism and culture conditions. *Mucor racemosus* (*M. lusitanicus*) ATCC 1216B was used in all experiments. The asexual sporangiospores designated C- and M-spores were produced on two types of media as described previously (Tripp & Paznokas, 1981, 1982). Sporangiospores were harvested 5 d after inoculation, washed twice in distilled water and counted with either a haemocytometer or a Coulter counter, and used as the inoculum.

Germination test media. The minimal germination medium (MGM) was a modification of the MSM in which L-glutamate and agar were omitted and D-glucose was replaced by other substrates at the various concentrations noted. Portions (10 ml) of MGM contained in 50 ml flasks were inoculated to a final concentration of 1×10^6 sporangiospores ml^{-1} and incubated at 25 °C in a gyratory water bath incubator shaking at 150 rev. min^{-1} .

Initiation of spore germination. Dormant *M. racemosus* sporangiospores average 20 μm^3 . Upon treatment with a suitable germinant, an increase in volume can be observed with a Coulter counter (model ZB1) and Channelyzer (model II) as described previously (Tripp & Paznokas, 1981). For these studies, spores that had increased to a volume $>45 \mu\text{m}^3$ were considered to have initiated germination.

Uptake and incorporation of L-glutamate and L-leucine. The uptake of L-glutamate and L-leucine by M- and C-spores incubated under permissive or non-permissive germination conditions was measured by following the accumulation of L-[U- ^{14}C]glutamate (1 mM; 1 $\mu\text{Ci } \mu\text{mol}^{-1}$, 37 kBq μmol^{-1}) or L-[U- ^{14}C]leucine (0.5 mM; 1 $\mu\text{Ci } \mu\text{mol}^{-1}$, 37 kBq μmol^{-1}). Spores ($5 \times 10^6 \text{ ml}^{-1}$) in MGM (pH 5.0 except where noted) containing the ^{14}C -labelled amino acid and the appropriate addition (glucose, 3-OMDG, L-glutamate or none as noted) were incubated in a gyratory water bath shaker at 25 °C. Samples (0.5 ml) were collected by vacuum filtration on a Millipore membrane filter (0.45 μm , HAWP) and washed with 10 ml of ice-cold unlabelled medium. The filters were counted as described previously (Tripp & Paznokas, 1982), and the results are reported as nmol accumulated per 10^6 spores. The incorporation of L-glutamate or L-leucine was measured by incubating the spores as described above, and then subjecting samples (1.0 ml) to a 30 min trichloroacetic acid treatment (5%, w/v, final concentration) at 4 °C. The samples were collected by vacuum filtration and washed with 10 ml of 5% trichloroacetic acid and 4 ml of 70% ethanol, both at 4 °C. The filters were counted as described above and the results are presented as nmol incorporated per 10^6 spores.

Determination of free amino acid pools in dormant spores. Three separate preparations of C- and M-spores were harvested, washed twice with water and counted with a Coulter counter. Each spore preparation was then suspended in 4 ml of 0.1 M-HCl (5×10^8 spores ml^{-1}) and subjected to five cycles of freezing and thawing using liquid nitrogen. The suspensions were pelleted (10000 g, 30 min, 4 °C) and the top 2 ml of the supernatants were taken and lyophilized. The samples were resuspended in 750 μl of buffer and an amino acid analysis (Beckman amino acid analyser, model 121) was performed on a portion of each sample. The results are presented as nmol amino acid per 10^9 spores.

Endogenous carbon loss during germination. Total organic carbon in spores incubated under germinating and non-germinating conditions was measured. Samples (10 ml) of spores ($5 \times 10^6 \text{ ml}^{-1}$) incubated in MGM/3-OMDG (20 mM) were collected by centrifugation (4000 g, 4 °C) and the pellets were washed once with glass-distilled water. The pellets were resuspended in glass-distilled water and the spore titre was determined with a Coulter counter. Carbon was determined using a total organic carbon analyser (Beckman model 915B) as described by the manufacturer. The results are presented as μg organic carbon per 10^6 spores.

Determination of sporangiospore protein. Samples (10 ml) of spores ($5 \times 10^6 \text{ ml}^{-1}$) incubated as noted in Table 3 legend were collected by centrifugation and washed once with glass-distilled water. The pellets were resuspended in glass-distilled water and counted with a Coulter counter. NaOH (final concentration 1 M) was added to the spore suspensions and heated to 100 °C for 20 min. Protein determinations were performed by the Lowry method.

Chemicals. ^{14}C -labelled L-glutamate and L-leucine were purchased from New England Nuclear. The glucose analogue 3-O-methyl-D-glucose (3-OMDG) was used at 20 mM and was purchased from Aldrich Chemical Co. Cyclic AMP was used at 10 mM and amino acids were usually 100 mM except where noted. These and all other chemicals were reagent grade and purchased from Sigma.

RESULTS

Germination induced by amino acids

A number of amino acids and combinations of amino acids were tested for their ability to induce germination of *Mucor racemosus* sporangiospores produced on either a minimal

Table 1. Germination of *M. racemosus* sporangiospores induced by L-amino acids and L-amino acid combinations

Germination test medium: addition(s) to MGM*	Percentage of spores >45 μm ³ by 8 h	
	M-spores	C-spores
L-Amino acids		
Glutamine (100 mM)	<5	59
Glutamate (100 mM)	<5	51
Proline (100 mM)	<5	26
Aspartate (100 mM)	<5	22
Alanine (100 mM)	<5	10
Histidine (100 mM)	<5	6
Phenylalanine (100 mM)	<5	6
Leucine (100 mM)	<5	5
Valine (100 mM)	<5	5
Glycine (100 mM)	<5	5
Others† (100 mM)	<5	<5
L-Amino acid combinations		
Glutamate (50 mM)	65	
Glutamine (10 mM)		
Proline (10 mM)		
Arginine (10 mM)		
Histidine (10 mM)	44	
Glutamate (50 mM)		
Proline (50 mM)		
Glutamate (50 mM)	18	
Histidine (50 mM)		
Control		
20 L-Amino acids‡ (each 10 mM)	>95	>95
Casamino acids (1%)	>95	>95
Peptone (1%)	>95	>95
3-OMDG (20 mM)	<5	65

* The pH of all L-amino acids test media was adjusted to 5.0.

† Singly: methionine, threonine, hydroxy-L-proline, aspartate, lysine, arginine, isoleucine, tyrosine, tryptophan, cysteine and cystine.

‡ In combination: all the amino acids tested singly.

(M-spores) or a complex (C-spores) sporulation medium. No single amino acid induced M-spore germination. Variable percentages of C-spores were induced to germinate by some, but not all of the amino acids (Table 1). A mixture of 20 L-amino acids induced greater than 95% of both M- and C-spores to germinate (Table 1). Amino acid mixtures representing the six major groups [in terms of where their carbon skeletons enter the tricarboxylic acid (TCA) cycle, i.e. at α -ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, acetoacetyl-CoA or acetyl-CoA] were added to MGM/glutamate medium and tested for their ability to induce M-spore germination. The mixture of amino acids which enter the TCA cycle through α -ketoglutarate (Glu, Gln, Pro, Arg, His) induced a high level of M-spore germination. The tandem mixtures of glutamate and proline or glutamate and hydroxy-L-proline were almost as effective (Table 1). Other tandem mixtures among this group (Glu, Gln, and Glu, Arg) as well as mixtures among the other five groups [Ile, Val, Met (14%); Phe, Tyr (11%); Asp, Asn (<5%); Leu, Lys, Trp (11%); Ala, Thr, Gly, Ser, Cysteine (<5%)] were not effective inducers of M-spore germination.

Free amino acid pools in sporangiospores

Since peptone, Casamino acids and certain amino acid combinations supported M-spore germination, where single amino acids did not (Table 1), the free amino acid pools in M- and C-spores were analysed to determine if qualitative or quantitative differences existed. Both C-

Table 2. *Free amino acid pools in M. racemosus sporangiospores*Results are the average values $\pm$ standard deviation for three separate sporangiospore preparations.

Compound	Concentration (nmol per 10^9 spores)		Concentration ratio (C-spores/M-spores)
	C-spores	M-spores	
Glutamate	627 $\pm$ 54.1	686 $\pm$ 126.5	
Glutamine	1188 $\pm$ 251.7	1077 $\pm$ 510.0	
Arginine	165 $\pm$ 9.0	212 $\pm$ 81.7	
Histidine*	272 $\pm$ 14.8	119 $\pm$ 8.8	2.3
Proline	291 $\pm$ 45.8	241 $\pm$ 99.7	
Hydroxy-L-proline*	1608 $\pm$ 325.4	11 $\pm$ 12.1	138
Isoleucine	21 $\pm$ 1.4	29 $\pm$ 6.4	
Valine	100 $\pm$ 15.1	112 $\pm$ 14.1	
Methionine*	22 $\pm$ 6.3	2 $\pm$ 0.39	7.8
Phenylalanine	19 $\pm$ 3.1	16 $\pm$ 3.7	
Tyrosine*	29 $\pm$ 7.4	14 $\pm$ 2.3	2.1
Aspartate	135 $\pm$ 27.8	174 $\pm$ 23.6	
Asparagine*	810 $\pm$ 184.8	463 $\pm$ 109.8	1.8
Leucine	20 $\pm$ 4.7	24 $\pm$ 6.0	
Lysine	84 $\pm$ 14.0	76 $\pm$ 6.0	
Alanine	436 $\pm$ 47.8	388 $\pm$ 32.4	
Threonine*	95 $\pm$ 21.8	49 $\pm$ 9.1	1.9
Glycine	132 $\pm$ 8.9	154 $\pm$ 5.5	
Serine*	245 $\pm$ 20.3	129 $\pm$ 24.9	1.9
Cysteine	30 $\pm$ 3.8	32 $\pm$ 6.1	
Ornithine	24 $\pm$ 9.1	18 $\pm$ 0.4	
α -Aminoadipic acid*	34 $\pm$ 5.4	17 $\pm$ 3.9	2.0
γ -Aminobutyric acid	87 $\pm$ 40.2	26 $\pm$ 21.0	
Cystathione	3 $\pm$ 1.6	5 $\pm$ 1.3	
Phosphoserine*	8 $\pm$ 5.8	20 $\pm$ 2.4	0.43
Phosphoethanolamine*	Not detected	293 $\pm$ 81.4	
NH ₃ *	608 $\pm$ 114.3	180 $\pm$ 30.0	3.4

* Compounds which varied significantly in concentration between C- and M-spores.

Fig. 1. Effect of pH on C-spore and M-spore germination ability in MGM/glutamate (O) or MGM/glutamate plus 20 mM-3-OMDG (●).

and M-spores contained all 20 amino acids but C-spores had significantly higher amounts of methionine, histidine, tyrosine, asparagine, threonine, serine and hydroxy-L-proline (Table 2), particularly hydroxy-L-proline (138-fold higher) and methionine (7.8-fold higher). Several other amino compounds were detected and C-spores contained significantly more α -aminoadipic acid and NH₃. M-spores, however, contained more phosphoserine (2.3-fold

Fig. 2. Effect of 3-OMDG and cycloheximide on the accumulation (O----O) and incorporation (●——●) of glutamate at pH 3.0 by C-spores: (a) MGM plus 20 mM-3-OMDG, pH 3.0 (permissive germination conditions); (b) MGM, pH 3.0 (non-permissive); (c) MGM plus 20 mM-3-OMDG and 300 g cycloheximide ml⁻¹, pH 5.0 (non-permissive).

higher). Phosphoethanolamine was found in M-spores but was not detected in C-spores (Table 2).

Effect of pH on spore germination

Greater than 95% of M- and C-spores germinated at similar rates in MGM/glucose (100 mM) or MGM/peptone (1%) throughout the pH range 3–9. M-spores did not germinate in MGM/glutamate at any pH (3–9) unless 3-OMDG was also added to the medium (Fig. 1). C-spores did not germinate in MGM/glutamate at pH 3, 8 or 9. The addition of 3-OMDG restored a high level of germination (Fig. 1) except at pH 9.

Uptake of glutamate

While M-spores were unable to initiate germination in MGM/glutamate at any pH, a percentage (50–75%) of C-spores could germinate on glutamate at pH 5.0, but not pH 3.0. The addition of glucose or 3-OMDG restored germination to >80% for all of the above conditions (Tripp & Paznokas, 1981). One explanation is that these non-permissive germination conditions reflect the inability of the spores to take up glutamate. If so, one should find different uptake kinetics for permissive and non-permissive conditions. No significant difference was observed in the kinetics of glutamate uptake under any of the following permissive or non-permissive conditions: for M-spores – MGM, MGM/3-OMDG, MGM (pH 3.0), MGM/3-OMDG (pH 3.0) were non-permissive conditions and MGM/glucose was a permissive condition; for C-spores – MGM and MGM (pH 3.0) were non-permissive conditions and MGM/glucose, MGM/3-OMDG, MGM/3-OMDG (pH 3.0) were permissive conditions.

Accumulation and incorporation of glutamate and leucine

Although the initial rates of glutamate uptake were the same for both germinating and non-germinating spores, the amounts of glutamate (Fig. 2) and leucine (Fig. 3) accumulated

Fig. 3. Effect of various permissive and non-permissive germination conditions on the accumulation (---) and incorporation (—) of leucine by C-spores: (a) C-spores in MGM (○, ●; non-permissive) and MGM plus 20 mM-3-OMDG (△, ▲; permissive); (b) M-spores in MGM (○, ●; non-permissive) and MGM plus 20 mM-3-OMDG (△, ▲; non-permissive); (c) C-spores in MGM/glutamate (100 mM) (○, ●; permissive) and MGM/glutamate plus 20 mM-3-OMDG (△, ▲; permissive); (d) M-spores in MGM/glutamate (100 mM) (○, ●; non-permissive) and MGM/glutamate plus 20 mM-3-OMDG (△, ▲; permissive).

Fig. 4. Effect of 3-OMDG on total organic carbon content of M-spores and C-spores. Spores were incubated in MGM (●) or MGM plus 20 mM-3-OMDG (○). Carbon was determined as described in Methods.

and incorporated were much greater under permissive germination conditions than non-permissive conditions. Incorporation but not accumulation was completely inhibited by cycloheximide (Fig. 2c).

Table 3. Relationship between 3-O-methyl-D-glucose-triggered germination and total spore protein

Spores were either (1) harvested and washed as described in Methods and stored frozen until needed for the protein determination, or (2) aerated by shaking at 25 °C in MGM for 5 h, harvested by centrifugation, washed twice in water and stored frozen, or (3) treated as in (2) except 20 mM-3-OMDG was added to the MGM. Results show the average value $\pm$ standard deviation for four replicates.

Treatment	Total protein (μg per 10^6 spores)	
	C-spores	M-spores
1. None	10.5 ± 0.83	6.8 ± 0.04
2. MGM	12.1 ± 1.9	7.1 ± 0.48
3. 3-OMDG	5.6 ± 0.41	6.7 ± 0.36

Fig. 5

Fig. 6

Fig. 5. Changes in protein content during germination of C-spores incubated under a variety of conditions: MGM plus 20 mM-3-OMDG; MGM plus 20 mM-3-OMDG and 100 mM-glutamate; MGM plus 100 mM-glutamate; or MGM plus 100 mM-glucose.

Fig. 6. Stimulatory effect of cyclic AMP on protein loss in C-spores initiated by 3-OMDG: spore swelling (a) and protein (b) in MGM plus 10 mM-cyclic AMP (Δ); MGM plus 20 mM-3-OMDG ($\circ$); or MGM plus 10 mM-cyclic AMP and 20 mM-3-OMDG ($\bullet$).

Effect of 3-O-methyl-D-glucose on total sporangiospore carbon

C-spores treated with 3-OMDG, a glucose analogue which is not metabolized by *M. racemosus* spores (Tripp & Paznokas, 1982), initiate germination and increase in volume to 70–90 μm^3 . Germ tubes do not appear until the cells achieve a volume of approximately 130 μm^3 . One explanation is that only C-spores contain endogenous reserves which are metabolized in response to the germination signal (i.e. 3-OMDG). Total organic carbon analysis of M- and C-spores supports this notion (Fig. 4). C-spores treated with 3-OMDG showed a decrease in carbon in an apparent inverse relationship to increases in spore volume. Both carbon loss and swelling ceased by 6–8 h and no further changes were observed even after 24 h incubation. Treatment of M-spores with 3-OMDG did not influence the total carbon content (Fig. 4) during 24 h.

Relationship between 3-O-methyl-D-glucose-triggered germination and total spore protein

Total protein was determined for C- and M-spore preparations before and after treatment with 3-OMDG. Freshly harvested C-spores contained nearly twice as much protein as M-spores (Table 3). C-spores triggered by 3-OMDG increased in volume to about $65\ \mu\text{m}^3$ by 5 h but decreased in total protein by about one-half (Table 3). M-spore protein was not quantitatively affected by 3-OMDG even after 24 h incubation.

Protein content of germinating spores

Protein synthesis is required for germination in most, if not all, fungal spore systems. This also appears to be the case for *M. racemosus* since cycloheximide completely blocks germination under permissive incubation conditions (Tripp & Paznokas, 1982; Orłowski & Sypherd, 1978). However, C-spores incubated under a variety of permissive conditions showed a decrease in total protein (Fig. 5). Treatment of C-spores with 3-OMDG (no carbon source) resulted in a sustained protein loss which was proportional to the degree of swelling; removal of 3-OMDG resulted in an immediate cessation of both protein loss and swelling. Treatment of C-spores with 3-OMDG + cyclic AMP stimulated the rate of protein loss. Also, the degree of C-spore development after 20 h incubation in 3-OMDG + cyclic AMP was significantly less ($48\ \mu\text{m}^3$) than in the same C-spores incubated in 3-OMDG alone ($88\ \mu\text{m}^3$) (Fig. 6). Treatment of M-spores with 3-OMDG did not affect their total protein content (Table 3).

DISCUSSION

As previously reported (Tripp & Paznokas, 1981), greater than 95% of M- and C-spores of *M. racemosus* germinate in MGM containing glucose, peptone or Casamino acids; but no M-spores and only a portion of C-spores germinate in the presence of glutamate or alanine. One explanation is that only certain amino acids can signal germination of *M. racemosus* M-spores as do only certain carbohydrates (Tripp & Paznokas, 1982). Germination of *Bacillus megaterium* spores, for example, is triggered by glucose and certain glucose analogues and also by proline and proline analogues but not by various other carbohydrates or amino acids (Rossignol & Vary, 1979; Vary, 1978). No single amino acid induced *M. racemosus* M-spore germination and only glutamate and glutamine induced 50% or more of C-spores to germinate (Table 1). A specific combination of amino acids present in peptone may be acting in concert as a germination signal analogous to glucose. To test this hypothesis several amino acid mixtures were used. Only combinations of amino acids which enter the TCA cycle through α -ketoglutarate induced significant M-spore germination. The combinations of glutamate and proline, or glutamate and hydroxy-L-proline were particularly effective.

The ability of certain amino acid combinations to trigger M-spore germination prompted us to examine the free amino acid pools in M- and C-spores since one or more of the amino acids of the α -ketoglutarate family may have been absent or low in M-spores. Similar amounts were found for most amino acids in both M- and C-spores (Table 2). Two qualitative exceptions were noted. First, extremely high amounts of hydroxy-L-proline existed in C-spores, but it was barely detectable in M-spores. Secondly, a high amount of phosphoethanolamine was present in M-spores, but was not detected in C-spores. The biological significance of these findings is unknown. Very high amounts of proline relative to the other amino acids accumulate in sporulating cells of *Saccharomyces cerevisiae*, *S. oleaceus* and *S. mellis* (Ramirez & Miller, 1964). These authors suggested that proline may not be metabolized during sporulation but preferentially used as an endogenous reserve during germination, or may play a role in the maintenance of dormancy. Hydroxy-L-proline and phosphoethanolamine may perform one or more of these roles in *M. racemosus*, or alternatively their presence may simply be a result of the different M- and C-sporulation conditions.

Since all 20 amino acids are found in *M. racemosus* spores, amino acids should not be limiting protein synthesis. Therefore, we examined protein synthesis in dormant and germinating spores. Only germinating spores incorporated significant amounts of glutamate or leucine into trichloroacetic acid precipitable material (Figs 2 and 3). Cycloheximide completely blocked this incorporation. These results demonstrate that dormant spores are not synthesizing protein even though substantial amino acid pools exist (Table 2). These amino acids could, however, be sequestered in some type of storage vesicle.

3-OMDG triggers C-spores to initiate germination and increase in volume by four- to five-fold (Tripp & Paznokas, 1981). Protein synthesis is required for this volume increase. Under these conditions spore development is sustained solely by endogenous reserves. Utilization of fungal spore endogenous reserves during the initial stages of germination is not unique to *M. racemosus*, for example, dry mass and specific gravity of the microconidia of *Trichophyton mentagrophytes* decrease significantly during germination (Hashimoto *et al.*, 1972). Since exogenously supplied amino acids induce *M. racemosus* spore germination, it is difficult to understand how the spore could maintain dormancy and contain free amino acid pools at the level that would be required to function as an endogenous reserve. On the other hand, it would be to the advantage of the spore to maintain a preformed supply of amino acids.

The ability of protein to function as an endogenous amino acid reserve has been demonstrated in several fungal systems including *Aspergillus* (Zonneveld, 1980), *Blastocladiella* (Lodi & Sonneborn, 1974), *Dictyostelium* (Cleland & Coe, 1969) and *Podospira* (Begueret & Bernet, 1973). Hence, we speculated that the endogenous reserve in *Mucor racemosus* spores might be protein. This notion was supported by the following observations. First, freshly harvested C-spores contained about twice as much protein as M-spores. Secondly, triggering of C-spores with 3-OMDG resulted in a sustained protein loss of about 50%, to the level of freshly harvested M-spores (Table 3). Thirdly, M-spore total protein was not affected by 3-OMDG treatment. Interestingly, the addition of exogenous cyclic AMP stimulated the rate of 3-OMDG-triggered protein loss in C-spores but did not initiate it (Fig. 6), and may explain why the rate of swelling but not the percentage of a C-spore population germinating on glutamate was stimulated by cyclic AMP (Wertman & Paznokas, 1981; Tripp & Paznokas, 1981). The observation that cyclic AMP stimulates the rate of protein degradation is not unique to *M. racemosus* spore germination. Increasing cyclic AMP concentrations correspond to increasing protease activity during the differentiation of *Aspergillus nidulans* under conditions where endogenous protein reserves supply the carbon and energy source (Zonneveld, 1980). Fourthly, removal of 3-OMDG resulted in an immediate cessation of both protein loss and spore swelling. The necessity for the continuous presence of 3-OMDG (Tripp & Paznokas, 1982) supports the hypothesis that it acts as a positive effector molecule. Conceivably, 3-OMDG may interact directly with a protease or act indirectly through a regulatory protein. An initial protein loss was also observed in C-spores germinating on glutamate, demonstrating that it is not unique to 3-OMDG-triggered germination (Fig. 5).

It appears that pH can influence the germination of *M. racemosus* sporangiospores. C-spores did not germinate on glutamate at pH 3 or 8 except when 3-OMDG, glucose or peptone was present (Fig. 1). This would suggest that there might be a pH-dependent conversion of glutamate to an initiation signal molecule(s). M-spores did not germinate on glutamate regardless of pH except in the presence of 3-OMDG, glucose or peptone. These data suggest that two germination trigger mechanisms exist in *M. racemosus* spores: (I) a pH-independent pathway present in both M- and C-spores and (II) a pH-dependent pathway apparently existing only in C-spores. One explanation for these observations is that compounds other than glucose may be excluded from spores depending on pH and spore type and that glucose may trigger uptake of these compounds. In disagreement with this hypothesis, the initial rates of glutamate uptake for M- and C-spores placed into permissive

pH-INDEPENDENT PATHWAY

pH-DEPENDENT PATHWAY

Fig. 7. Hypothetical mechanism by which *Mucor racemosus* sporangiospores initiate germination.

(glucose or 3-OMDG) or non-permissive (MGM or glutamate, pH 3) germination conditions were the same (data not shown). Similar results were obtained with leucine (Fig. 3) and lysine (data not shown).

Alternatively, glucose may 'turn on' macromolecular synthesis either by direct interaction with a regulatory protein, or indirectly by signalling the mobilization of endogenous reserves, resulting in an intracellular flood of biosynthetic intermediates. According to this hypothesis M-spores would lack the endogenous reserves necessary for energy production or biosynthesis. As mentioned above, M-spores contained about one-half the protein of C-spores. This could explain why M-spores were not triggered by the non-metabolizable glucose analogue 3-OMDG unless a carbon source was present.

From the information presented here, we propose the following model for the regulation of dormancy in *M. racemosus* sporangiospores (Fig. 7). For the pH-independent pathway, we propose that the glucose molecule interacts indirectly with a regulatory protein or directly with a protease to activate it. The activated protease degrades the endogenous protein reserve supplying a flood of amino acids which are metabolized as an energy source or utilized for macromolecular synthesis. Further, we propose that it is this sudden increase in free amino acids which triggers protein synthesis and is responsible for the synchronous germination on glucose and 3-OMDG. Supplying a mixture of amino acids exogenously (e.g. peptone) circumvents the requirement for glucose. This may explain why Orłowski & Sypherd (1978) did not see a net protein loss in *M. racemosus* spores germinating on a yeast extract, peptone and glucose medium.

Evidence for the pH-dependent pathway only exists for C-spores. We propose that this is the pathway by which triggering occurs by non-hexoses. The non-hexose must be metabolized (e.g. via the TCA cycle) to supply the carbon skeletons for amino acid interconversion. When the free amino acid pools reach a critical concentration, germination is initiated. It is probable that the conversion of the non-hexose to one or more specific amino acids is the pH-dependent step. An alternative would be that non-hexoses would have to be converted to glucose which then would initiate germination.

The mechanism by which a compound triggers germination in a dormant spore is unknown. Numerous models have been proposed (Dring & Gould, 1975; Prasad *et al.*, 1972; Vary, 1978; Woese *et al.*, 1968). Our studies suggest that the control over dormancy in *M. racemosus* sporangiospores is at the level of protein metabolism – both degradation and

synthesis. Compounds which trigger protein degradation (3-OMDG) or protein synthesis (amino acids) result in the breaking of the dormant state.

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