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一、中文摘要

在過去二十年中，因愛滋病毒感染，組織或器官移植以及癌症化學治療等原因，所導致免疫系統缺陷之個體大量增加，因而使得真菌性感染成為新興之醫療問題。隱球菌(*Cryptococcus neoformans*) 為一伺機性人體病原真菌，對免疫缺陷之族群，具致命性真菌性腦膜炎之威脅。隱球菌之病原性研究顯示，其主要致病因子包括，莢膜(capsule)、黑色素(melanin)，以及該菌之生殖型基因位點(mating type locus)。

經由自然環境及臨床的調查，以及動物病原性的實驗等結果顯示，交配型 *á* 基因位點可能與環境流行及致病性有關。據此，交配型 *á* 基因位點被選殖出，其上之基因已被陸續分析，其中包含三個費洛蒙基因。經分析三個費洛蒙基因之突變株後發現，在交配型 *á* 的菌株中，可能存有費洛蒙自我調控之機轉存在，並可能為交配型 *á* 的菌株具較高病原性之成因。本計畫之目的乃在進一步瞭解隱球菌血清型 D 型菌之費洛蒙反應機制，探討交配型基因位點與致病機制及其他生理調控之關係，主要分為兩個方向，一為針對隱球菌血清型 D 型菌之 G 蛋白質複合體β次單元，進行其生理角色之探討，二為找出費洛蒙反應機制之下游基因 *STE6*，以進一步確認費洛蒙自我調控之機轉與有性生殖、致病性之關係。

90 年度為執行第一年，在血清型 D 型隱球菌之 G 蛋白質複合體β次單元分析上，已篩選及確認 G 蛋白質β次單元基因變異種。性狀分析顯示，其參予有性生殖及單價菌絲生長之調控，至於是否參予調控致病性，則有待建構好之變異種回復菌株後，進行動物試驗後始能確認。在尋找費洛蒙反應機制之下游基因 *STE6* 上，已自史丹福大學隱球菌基因體計劃中確認，*STE6* 基因變異株亦已篩選獲得，初步分析之結果顯示，其亦參予有性生殖之調控，而在單價菌絲生長及致病性上之角色，有待進一步證明。另外，在測試與調整細胞生長及 RNA 分離等條件及步驟後，subtraction PCR 篩選其他參與訊息傳導基因的實驗，亦將於近期進行。

關鍵詞：隱球菌、費洛蒙反應機制、G 蛋白質β次單元、費洛蒙傳送蛋白質

Abstract

Fungal infection has drawn lots of attention due to dramatically increased number of immunocompromised patients caused by HIV infection, organ and tissue transplantation, and cancer chemotherapy in the past two decades. *Cryptococcus neoformans*, a human pathogenic basidiomycetous yeast, causes the life-threatening meningoencephalitis mainly in such individuals with compromised immune functions. Studies of the pathogenesis in *C. neoformans* have revealed several important virulence factors such as capsule, melanin, and interestingly mating type locus. Environmental and clinical prevalence of *MAT*α strains and virulence

studies of the congenic pair of *C. neoformans* serotype D strains have suggested that *MAT* α locus might involve in regulating the virulence in *C. neoforman*. Following these observations, *MAT* α locus has been identified and characterized. Three copies of pheromone precursor genes were identified in the *MAT*α locus. Characterization of three pheromone genes deletion mutant strains have suggested an autocrine signaling loop may function and contribute to the virulence of the *MAT*å cells (Shen *et al*., 2002).

The purpose of this proposal is to characterize the components in the pheromone response pathway of *C. neoforman* serotype D strain and further address how mating type locus regulates the virulence and autocrine signaling loop functions in *C. neoforman*.

C. neoformans serotype D strain *GPB1* gene were identified and disrupted in the *MATa* strain. *MATa gpb1* mutants were also identified in the progeny of cross between the *MAT*α *gpb1* mutant and *MATa* strain. The *MAT*α *gpb1* and *MATagpb1*mutant strains were mating impaired but not sterile when coincubated with the wild-type strain of opposite mating type on V8 mating medium. Haploid fruiting was reduced, but not completely abolished, in the *MAT*α *gpb1* mutant strains, similar to the *mf*α *1,2,3* pheromoneless mutant.

To further address how pheromones act in the autocrine signaling loop, we have identified *STE6* homologue, a pheromone transporter, in the *C. neoformans* genome project at Stanford Genome Technology Center and begun to dissect its function. By disrupting the *STE6*, we found that *ste6* mutants in either *MAT*å or *MAT*a background showed partially impaired mating function, although slight differences were noticed. However, when *ste6 MAT*å and *MAT*a mutants cross with each other, the mating process was nearly completely abolished. Our data indicates that the STE6 functions bilaterally and is required but not essential for mating in *C. neoformans*.

Currently we are constructing the *gpb1* and *ste6* reconstituted strains, virulence test will be conducted when verified those strains. We are also conducting epistasis and functional analysis on these two genes, and hoping to claify their role in virulence and other physiological processes.

To identify novel targets in the downstream of the signaling pathway, we have optimized growth conditions and RNA extraction procedures. We will start the subtractive PCR screen shortly.

Keywords: *Cryptococcus neoformans*,
pheromone response pathway,
heterotrimeric GTP binding
protein ß subunit,
pheromone transporter

二、緣由與目的

Infectious diseases have long been serious concerns over human public health. In the past two decades, human infections caused by the opportunistic fungal pathogens have dramatically increased due to the emerging number of individuals with immune compromised function. The impaired immune functions among these individuals were the consequences of human immuno- deficiency virus infection, cytotoxic chemotherapy for malignancies, immuno-suppression for organ transplantation, and long-term steroid treatment (Mitchell and Perfect, 1995).

Cryptococcosis is a pulmonary, systemic, or meningitic infection caused by the haploid basidiomycetous yeast, *Cryptococcus neoformans*. This fungus has drawn lots of attention due to several reasons. First, the incidence of cryptococcal infection has increased significantly due to the reasons just mentioned above. Second, life-long medical treatment is required for AIDS patients who have cryptococcal meningitis. Finally, the drug resistance to the currently used antifungal compounds has developed among *C. neoformans* isolates (Alspaugh *et al.*, 1998). Therefore, studying the pathways regulating the pathogenesis in *C. neoformans* will provide the opportunity to identify the novel antifungal target for practical therapeutic use.

In addition to the practical significances, ease manipulation and well developed genetic tools have led *C. neoformans* to a model system for studying pathogenesis and other basic physiological processes in pathogenic fungi. *C. neoformans* is a heterothallic basidiomycete with a bipolar mating system, MATá and MATa (Kwon-Chung, 1975; Alspaugh *et al.*, 2000). Under nitrogen limitation condition, strains of opposite mating type produce conjugation tubes and fuse to form filamentous dikaryon with fused clamp connections. The tips of the filaments differentiate to form basidium, where nuclear fusion and meiosis occur, and finally four long chains of basidiospores are produced. Besides nutritional signal, mating is also mediated via pheromone molecules (Shen *et al,* 2002). An asexual fruiting process, termed haploid or monokaryotic fruiting, only occurs in the MATá strains in response to nitrogen starvation and severe desiccation (Wickes *et al.*, 1996). This process occurs in the absence of MATa mating partner, but can be greatly enhanced by the adjacent MATa cells (Wang *et al.*, 2000). This MATá specific process may contribute to the prevalence of MATá strains in the natural and clinical isolates (Kwon-Chung and Bennett, 1978).

Studies of the pathogenesis in *C. neoformans* revealed several important factors: melanin formation and capsule production (Kwon-Chung and Rhodes, 1986), the ability to grow at 37℃ or higher temperature (Odom *et al.*, 1997), and mating type locus (Kwon-Chung *et al.,* 1992). The linkage of mating type á locus to the disease is particularly interesting. In addition that MATá strains are more prevalent than MATa strains in the environment, MATá strains are more virulent than congenic MATa strains in a murine model of systemic cryptococcal infection (Kwon-Chung *et al.,* 1992).

Following these interesting observations, mating type á locus was identified (Moore and Edman, 1993). Initial isolation of genomic region containing the MATá locus was done through difference cloning, and a region encoding the mating pheromone precursor gene was identified. Subsequent characterization of the flanking genomic region has expanded the locus to a ~71 kb genomic contig (Karos *et al.,* 2000; Lengeler *et al.*, unpub. results). Sequence analysis of the MATá locus found components in the pheromone response pathway such as *STE12á*, *STE11á, STE20á*, pheromone receptor gene, and three copies of pheromone precursor genes (Karos *et al.,* 2000; Lengeler *et al.*, unpub. Results; Shen *et al,* 2002).

C. neoformans pheromone gene, *MFá1*, was found to stimulate conjugation tube formation when transformed into MATa cells (Moore and Edman, 1993). The predicted *C. neoformans,* MFá1, MFá2, and MFá3, are similar to yeast *S. cerevisiae* a mating factor with a conserved CAAX prenylation motif at the carboxy-terminus (Moore and Edman, 1993; Davidson *et al.*, 2000; Shen *et al,* 2002). To determine the roles of *MFá* pheromone genes in *C. neoformans,* these genes are disrupted in serotype D strains. Phenotypic characterization of the triple pheromone deletion strains found that pheromones are not absolutely required for mating in *C. neoformans*. More surprisingly, deletion of all three pheromone genes also results a haploid fruiting defect. The reconstituted strain with wild-type copy of *MFá1* or overexpression of the *MFá1* gene enhances haploid fruiting in the triple deletion or wild-type strains. These results suggested that the MFá mating pheromone regulates the haploid fruiting possibly via an autocrine signaling pathway (Shen *et al,* 2002). This signaling loop may explain why the MATá strains prevail in the environment and why the MATá strains are inherently more virulent than the MATa congenic strains. It would be extremely interesting to dissect this autocrine signaling loop, determine whether secretion of the pheromone molecules is required for the induction, and if it is the case, what receptor is targeted by the loop.

Evidence from the study of *C. neoformans STE12á* homolog suggested the linkage between the mating type and virulence in *C. neoformans* serotype D strains (Wickes *et al.*, 1997; Chang *et al.,* 2000). Overexpression of the *C. neoformans*

STE12á stimulates haploid fruiting and the expression of *CNLAC1*, which is involved in the production of melanin (Wickes *et al.*, 1997). Gene disruption analysis of *STE12á* in both serotype A and D strains had drawn the conclusions that STE12á plays a minor role in mating but is required for haploid fruiting (Yue *et al.*, 1999; Chang *et al.,* 2000). Reporter gene analysis indicated that STE12á might also involve in regulating virulence, although the result is not very conclusive (Chang *et al.,* 2000). Virulence studies of serotype D wild-type strains and *ste12á* mutants showed deletion of *STE12á* results the low mortality rate and small *in vivo* capsule size. On the other hand, serotype A strains lacking of *STE12á* are fully virulent (Yue *et al.*, 1999). These studies suggested that the mechanisms regulating the virulence in *C. neoformans* serotype A and serotype D strains may be quite divergent. This argument was further strengthened by the comparison of a G protein á subunit, GPA1 and components in the cAMP-PKA signaling pathway among *C. neoformans* serotype A and serotype D strains (Allen *et al.,* unpub. Results; D'souza *et al.,* 2001).

In additional that mating type specific components were identified, pheromone response elements present in both mating types were also identified (Wang *et al.*, 2000). The gene encoding a heterotrimeric Guanine nucleotide binding protein â subunit, GPB1, was cloned in *C. neoformans* serotype A strains. Disruption of GPB1 resulted a sterile phenotype which suggested its active role in mating. This is analogous to the role of STE4 in *S. cerevisiae*. *gpb1* mutants also exhibited a haploid fruiting defect. Overexpression of the presumptive downstream components, a conserved MAP kinase *CPK1* or *STE12á,* in the pheromone response pathway suppressed mating or haploid fruiting defect respectively. These evidences supported the model that these components function in the same signaling pathway (Wang *et al.*, 2000).

Besides GPB1, the heterotrimeric G protein á subunits, GPA1, GPA2, and GPA3, were also identified and characterized (Alspaugh *et al.*, 1997;Allen *et al.,* unpub. Results, Shen *et al.,* unpub. results). GPA1 functions upstream of the cAMP-PKA pathway and regulates capsule and melanin production, mating and virulenc (Alspaugh *et al.*, 1997). GPA1 plays an important role in regulating mating and virulence in *C. neoforman* serotype A strains; however, the homolog in serotype D strains appears to play a very minor role in these processes (Allen *et al.,* unpub. Results).

Study of another G protein á subunit, GPA3, has revealed interesting findings. Deletion of *GPA3* in *C. neoforman* serotype D strains resulted a dramatic haploid fruiting phenotype in response to severe nitrogen and water deprivation after 3 to 5 days (Shen *et al.,* unpub. Results). This result suggested that GPA3 might form a heterotrimeric G protein complex with GPB1 and function in the upstream of the conserved MAP kinase module. The lack of GPA3 in the G protein complex results in the constitutive activation of the pathway by releasing the active GPB1 in response to nitrogen starvation and desiccation. It will be conclusive to conduct two-hybrid assay to detect the physical interaction between GPA3 and GPB1. It will be interesting to test the virulence of gpa3 mutant. Additionally, the constitutively active gpa3 mutant will be useful to set up a differential screen to isolate other downstream targets of the pathways involved in haploid fruiting, mating, and possibly virulence.

The proposed study is to further characterize the pheromone response pathway in *C. neoforman* serotype D strain. We will first determine the physiological roles of *GPB1* homolog from serotype D strain and compare with its correspondence in serotype A strain. We will also try to identify other components like STE6 in the pheromone response pathway to further dissect the interesting autocrine signaling loop. Finally, we would like to set up a differential screening approach using *gpa3* mutant to identify novel genes important for differentiation and virulence in this pathogenic fungus. To the end, we hope some of the genes can be exploited for novel antifungal therapy.

三、結果與討論

1. Disruption of *C. neoformans GPB1*

C. neoformans serotype D strain *GPB1* gene were identified in the *C. neoformans* genome project at Stanford Genome Technology Center. To determine the cellular functions of *GPB1* homolog in *C. neoformans* serotype D strains, the *GPB1* gene was replaced with a *gpb1::: URA5* mutant allele by biolistic transformation and homologous recombination in the *MAT*α *ura5* strain JEC43. Five transformants identified by PCR and confirmed by Southern blot contained the *gpb1* mutant allele and lacked the wild-type locus (Fig. 1). By crossing the *MAT*α *gpb1* mutant with *MATa* strain, *MATa gpb1* mutants were obtained and verified (Fig. 1).

The *MAT*α *gpb1* and *MATagpb1*mutant strains were mating impaired but not sterile when coincubated with the wild-type strain of opposite mating type on V8 mating medium (Fig. 2). Haploid fruiting was reduced, but not completely abolished, in the *MAT*α *gpb1* mutant strains, similar to the *mf*α *1,2,3* pheromoneless mutant.

Currently we are constructing the reconstituted plasmid, and the *GPB1* gene will be reintroduced into the *gpb1* 5-FOA strain we selected. The resulting transformants will be screened by PCR and verified by Southern blot analysis. The verified reconstituted strains will be side-by-side compared with the wild-type and *gpb1* deletion strains for the functions of mating, haploid fruiting. More importantly, the *MAT*α and *MATa gpb1* mutants will be subject to virulence study with respective reconstituted and wild-type strains to clarify its role in virulence.

To determine the functions of GPB1 related to other genes in the pheromone response pathway, the epistasis or overexpression analyses is ongoing. A heterotrimeric GTP binding protein *á* subunit, *GPA3*, was also identified and disrupted (Shen *et al.*, unpublished data). Based on the phenotype of *gpa3* mutant strains, GPA3 is thought to function in the upstream of pheromone response pathway and highly possible to physically interact with GPB1. Currently we are constructing *gpb1gpa3* double mutant and will also try to determine if these two proteins physically interact by two-hybrid assay. Overexpression of *GPB1* in the serotype D MATá and MATa wild-type strains will be conducting to determine what physiological functions might reside in the downstream pathway.

2. Identification of *C. neoformans STE6*

Virulence studies of the congenic pair of *C. neoformans* strains have shown that *MAT*á cells are more virulent than *MAT*a cells. Characterization of mating pheromone genes in the *MAT*á strains have suggested an autocrine signaling loop may function and contribute to the virulence of the *MAT*á cells (Shen *et al.*, 2002). To further address how pheromones act in the autocrine signaling loop, we have identified *STE6* homologue, a pheromone transporter, in the *C. neoformans* genome project at Stanford Genome Technology Center and begun to dissect its function. By disrupting the *STE6*, we found that *ste6* mutants in either *MAT*á or *MAT*a background showed partially impaired mating function, although slight differences were noticed. However, when *ste6 MAT*á and *MAT*a mutants cross with each other, the mating process was nearly completely abolished (Fig. 3). Our data indicates that the STE6 functions bilaterally and is required but not essential for mating in *C. neoformans*.

Similar to *GPB1* experiments, the *STE6* reconstituted plasmid was made and will be reintroduced into *ste6* mutant. The phenotypes of *ste6* mutant strains will be reconfirmed to determine if it plays roles in haploid fruiting or virulence.

Heterologous expression of *C. neoformans STE6* in *S. cerevisiae ste6* mutant will be conducting. Additionally, heterologous expression of *S. cerevisiae* a-factor in *C. neoformans mfá1,2,3* pheromoneless mutant and wild-type strain are ongoing to determine if *C. neoformans* STE6 transporter and lipopeptide processing machinery are functionally conserved across distantly related yeasts.

3. Subtraction PCR approach

To identify the novel genes acting downstream of the conserved MAP kinase cascade, we originally planned to set up a subtractive enrichment screen using *gpa3* mutant strain. Unfortunately due to low recovery of the cells from the culture medium, we are unable to produce enough RNA material for the procedure. To access other alternatives, we decided to use wild-type strains under various mating and growth conditions to conduct the subtraction experiment. Right now we have optimized conditions for good quality and high yield of RNA samples, and are further testing the quality using known genes in the pathways to confirm the RNA quality. Poly A RNA will be collected from the pools of high quality RNA and subtraction PCR procedure will start shortly.

In summary, the aims of project for the first year are accomplished. *STE6* part is ahead of schedule, we are hoping we can finalize the experiments of *GPB1* and *STE6* in the second year. If we got some interesting targets out of the subtraction screen, then we will continue to dissect their roles in differentiation and virulence of *C. neoformans.*

四、參考文獻

[1] Alspaugh, J. A., R. C. Davidson, and J. Heitman. 2000b. Morphogenesis of *Cryptococcus neoformans*, p. 217–238. *In* J. F. Ernst and A. Schmidt (ed.), Dimorphism in human pathogenic and apathogenic yeasts, vol. 5. Karger, Basel, Switzerland.

[2] Alspaugh, J. A., J. R. Perfect, and J. Heitman. 1998. Signal transduction pathways regulating differentiation and pathogenicity of *Cryptococcus neoformans*. Fungal Genet. Biol. 25:1–14.

[3] Alspaugh, J. A., J. R. Perfect, and J. Heitman. 1997. *Cryptococcus neoformans* mating and virulence are regulated by the G-protein a subunit GPA1 and cAMP. Genes Dev. 11:3206–3217.

[4] Chang, Y. C., B. L. Wickes, G. F. Miller, L. A. Penoyer, and K. J. Kwon-Chung. 2000. *Cryptococcus neoformans STE12á* regulates virulence but is not essential for mating. J. Exp. Med. 191:871–882.

[5] Davidson, R. C., T. D. Moore, A. R. Odom, and J. Heitman. 2000. Characterization of the MFá pheromone of the human fungal pathogen *Cryptococcus neoformans*. Mol. Microbiol. 38:1017-1026.

[6] D'souza, C. A., J. A. Alspaugh, C. Yue, T. Harashima, G. M. Cox, J. R. Perfect, and J. Heitman. 2001. Cyclic AMP-dependent protein kinase controls virulence of the fungal pathogen *Cryptococcus neoformans*. Mol. Cell. Biol. 21:3179-3191.

[7] Karos, M., Y. C. Chang, C. M. McClelland, D. L. Clarke, J. Fu, B. L. Wickes, and K. J. Kwon-Chung. 2000. Mapping of the *Cryptococcus neoformans MATá* locus: presence of mating type-specific mitogen-activated protein kinase cascade homologs. J. Bacteriol. 182:6222-6227.

[8] Kwon-Chung, K. J. 1975. A new genus, *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. Mycologia 67:1197–1200.

[9] Kwon-Chung, K. J., and J. E. Bennett. 1978. Distribution of á and a mating types of *Cryptococcus neoformans* among natural and clinical isolates. Am. J. Epidemiol. 108:337–340.

[10] Kwon-Chung, K. J., J. C. Edman, and B. L. Wickes. 1992. Genetic association of mating types and virulence in *Cryptococcus neoformans*. Infect. Immun. 60:602–605.

[11] Kwon-Chung, K. J., and J. C. Rhodes. 1986. Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. Infect. Immun. 51:218-223.

[12] Mitchell, T. G., and J. R. Perfect. 1995. Cryptococcosis in the era of AIDS—100 years after the discovery of *Cryptococcus neoformans*. Clin. Microbiol. Rev. 8:515–548.

[13] Moore, T.D., Edman, J.C. 1993. The alpha-mating type locus of *Cryptococcus neoformans* contains a peptide pheromone gene. *Mol. Cell. Biol.* 13:1962-1970.

[14] Odom, A., S. Muir, E. Lim, D. L. Toffaletti, J. Perfect, and J. Heitman. 1997. Calcineurin is required for virulence of *Cryptococcus neoformans*. EMBO J. 16:2576–2589.

[15] Shen, W. C., R. C. Davidson, G. M. Cox, and J. Heitman. 2002. Pheromones stimulate mating and differentiation via paracrine and autocrine signaling in *Cryptococcus neoformans*. Eukaryotic cell 1:366-377.

[16] Wang, P., J. R. Perfect, and J. Heitman. 2000. The G-protein b subunit GPB1 is required for mating and haploid fruiting in *Cryptococcus neoformans*. Mol. Cell. Biol. 20:352–362.

[17] Wickes, B. L., U. Edman, and J. C. Edman. 1997. The *Cryptococcus neoformans STE12á* gene: a putative *Saccharomyces cerevisiae STE12* homologue that is mating type specific. Mol. Microbiol. 26:951–960.

[18] Wickes, B. L., M. E. Mayorga, U. Edman, and J. C. Edman. 1996. Dimorphism and haploid fruiting in *Cryptococcus neoformans*: association with the á-mating type. Proc. Natl. Acad. Sci. USA 93:7327–7331.

[19] Yue, C., L. M. Cavallo, J. A. Alspaugh, P. Wang, G. M. Cox, J. R. Perfect, and J. Heitman. 1999. The STE12a homolog is required for haploid filamentation but largely dispensable for mating and virulence in *Cryptococcus neoformans.*

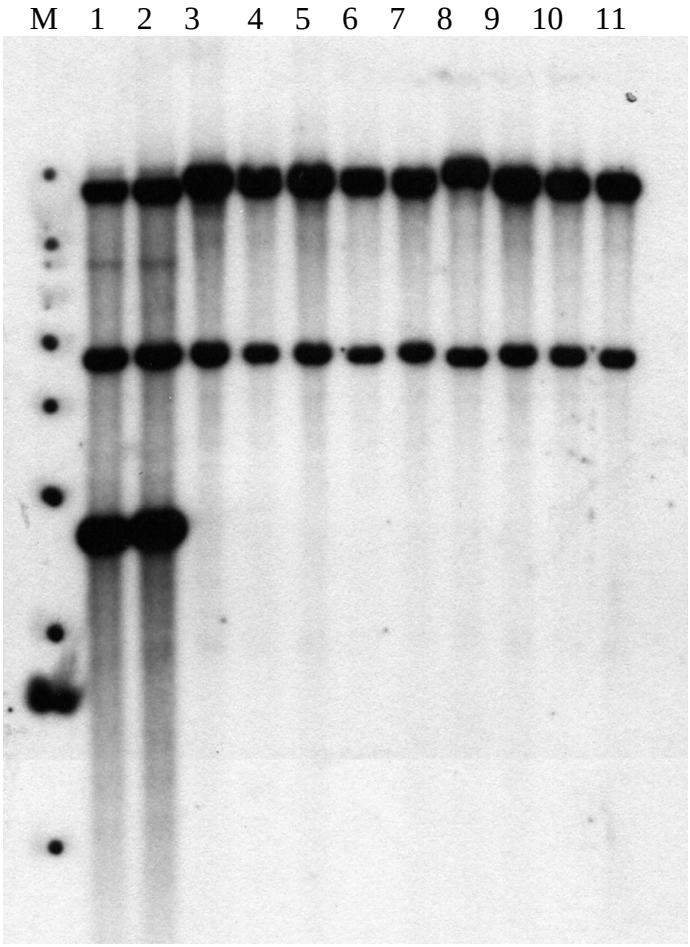
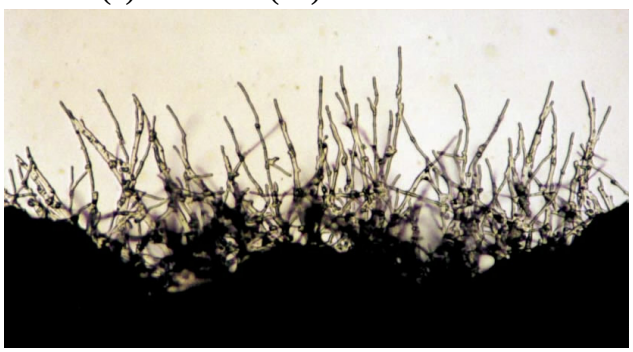
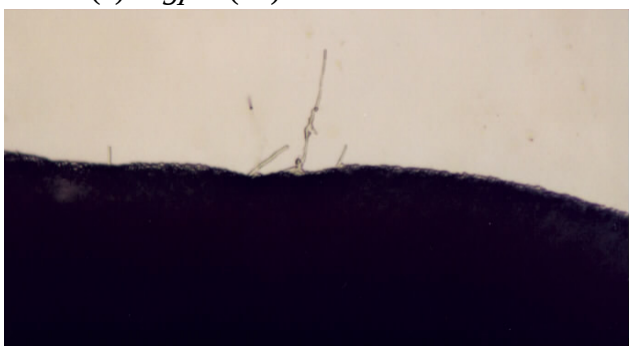


Fig. 1. Disruption of the *GPB1* gene. Genomic DNA of wild-type *MAT*α (lane 1, JEC21) and *MATa* (Lane 2, JEC20) strains, *MAT*α*gpb1* original transformants (Lane 3 to 7), and *MAT*α*gpb1* (Lane 8 and 9) and *MATagpb1* (Lane 10 and 11)progeny in the cross of *MAT*α*gpb1* original transformants and *MATa* strain was digested with *Eco*RI, electrophoresed, and analyzed by Southern hybridization with *GPB1* containing *Pst*I genomic fragment.

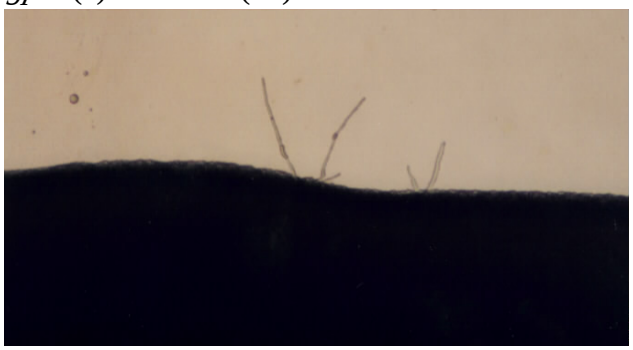
JEC20 (a) X JEC21 (α)



JEC20(a) X *gpb1*(α)



gpb1(a) X JEC21 (α)



JEC20 (a) X *gpb1*seroA (α)

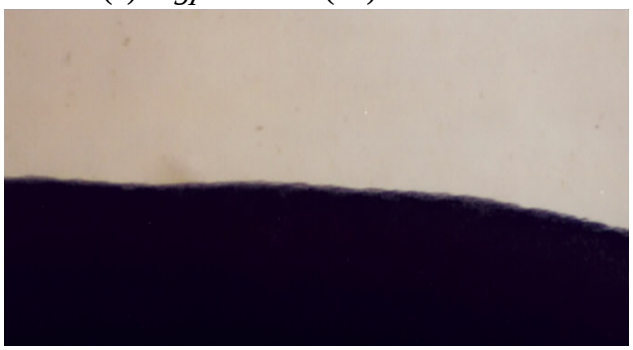
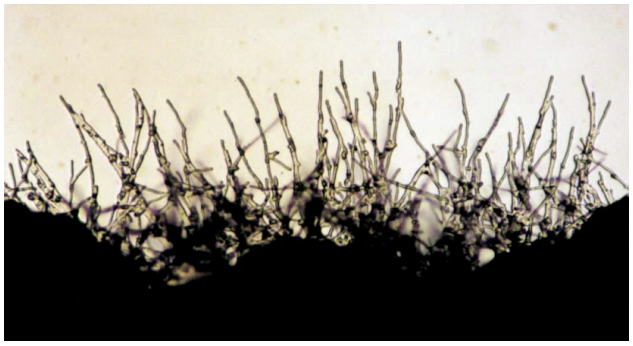
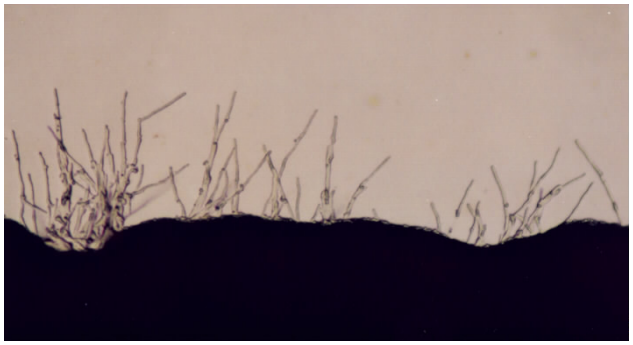


Fig. 2. The *MAT*α*gpb1* and *MAT*a*gpb1*mutant strains were mating impaired but not sterile. Wild type and *gpb1* strains were coincubated with mating partners on V8 mating plates in the dark for 3 days at 26℃. The edges of the mating mixtures were photographed at X100 magnification.

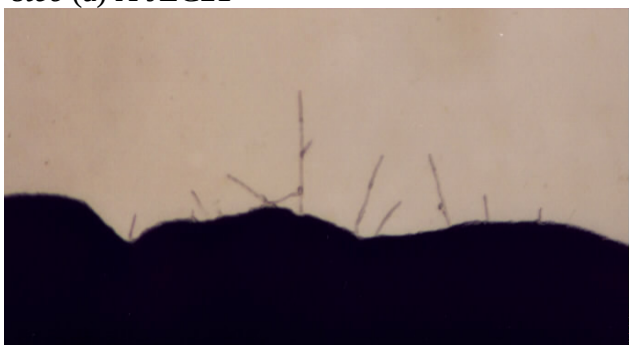
JEC20 (a) X JEC21 (α)



JEC20 (a) X *ste6* (α)



ste6 (a) X JEC21



ste6 (a) X *ste6* (α)

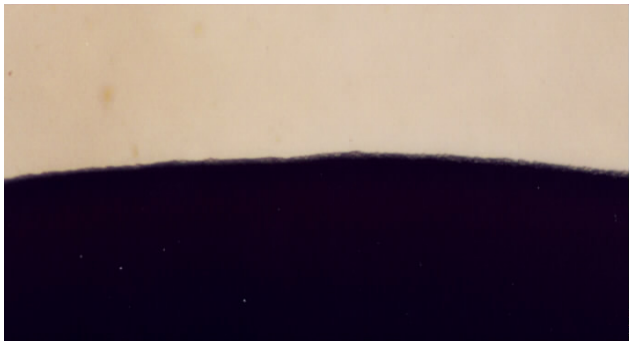


Fig. **3.** The STE6 functions bilaterally and is required but not essential for mating in *C. neoformans*. Wild type and *ste6* strains were coincubated with mating partners on V8 mating plates in the dark for 3 days at 26℃. The edges of the mating mixtures were photographed at X100 magnification.

附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

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※　人體病原真菌隱球菌生殖, 菌絲生長及致病分子機制之探討 ※

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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC90－2311－B－002－062

執行期間： 90 年 8 月 1 日至 91 年 7 月 31 日

計畫主持人：沈偉強

共同主持人：

計畫參與人員：薛雁冰

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：臺灣大學植物病理系

中　華　民　國　91　年　10　月　31　日