

Induction of signal transduction pathways related to the pathogenicity of *Cryptococcus neoformans* in the host environment

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Summary

Cryptococcus neoformans, a human pathogenic fungus, infects immunocompromised humans and causes serious diseases such as cerebral meningitis. *C. neoformans* controls the expression of virulence factors in response to the host environment via various signal transduction pathways. Understanding the molecular mechanisms involved in *C. neoformans* infection will contribute to the development of methods to prevent and treat *C. neoformans*-related diseases. *C. neoformans* produces virulence factors, such as a polysaccharide capsule and melanin, to escape host immunity. Several proteins of *C. neoformans* are reported to regulate production of the virulence factors. In this review, on the basis of studies using gene-deficient mutants of *C. neoformans* and animal infection models, we outline the signal transduction pathways involved in the regulation of virulence factors.

Keywords: *Cryptococcus neoformans*, fungus, host factor, infection, pathogenicity, signal transduction pathway

1. Introduction

Several fungi cause various serious infections in immunocompromised patients, such as those taking immunosuppressive drugs to receive organ transplants and AIDS patients. *Cryptococcus neoformans* is an opportunistic fungal pathogen that causes respiratory diseases and cerebral meningitis (1-4). Cerebral meningitis is highly lethal and a main cause of death in AIDS patients (3). *C. neoformans* infects humans through carrier animals such as pigeons, and enters into the human blood via the nasal passages or wound sites (5). Immune cells in the human body are involved in eliminating *C. neoformans*. To resist immune systems, *C. neoformans* produces virulence factors such as a polysaccharide capsule and melanin (6).

A polysaccharide capsule surrounds the *C. neoformans* cell, thereby preventing phagocytosis, blocking antigen presentation to T cells, suppressing inflammatory cytokine production, and depleting

complement proteins (Figure 1) (7,8). Melanin is a polymer produced via catecholamine oxidation that accumulates in the cell wall of *C. neoformans* (9). Melanin protects against the oxidants produced by immune cells and decreases the porosity of the cell wall (Figure 1) (9-12). The melanin-induced decreased porosity of the cell wall plays an important role in the cell wall barrier function against host immune systems (12). Both production of the capsule and melanin by *C. neoformans* contributes to its escape from host immunity (13). Gene-deficient *C. neoformans* mutants unable to produce the capsule and melanin exhibit reduced mouse-killing ability (14,15). Therefore, regulation of the capsule and melanin production by *C. neoformans* is important for controlling its pathogenicity (16,17).

C. neoformans controls the growth and expression of its virulence factors in response to the host environment (16,17). It adapts to the host environment by regulating the expression of various genes in response to changes in environmental components such as sugars, amino acids, and metals (18).

In this review, we outline the signal transduction pathways involved in the pathogenicity of *C. neoformans* and the host factors that affect the activation of these signal transduction pathways.

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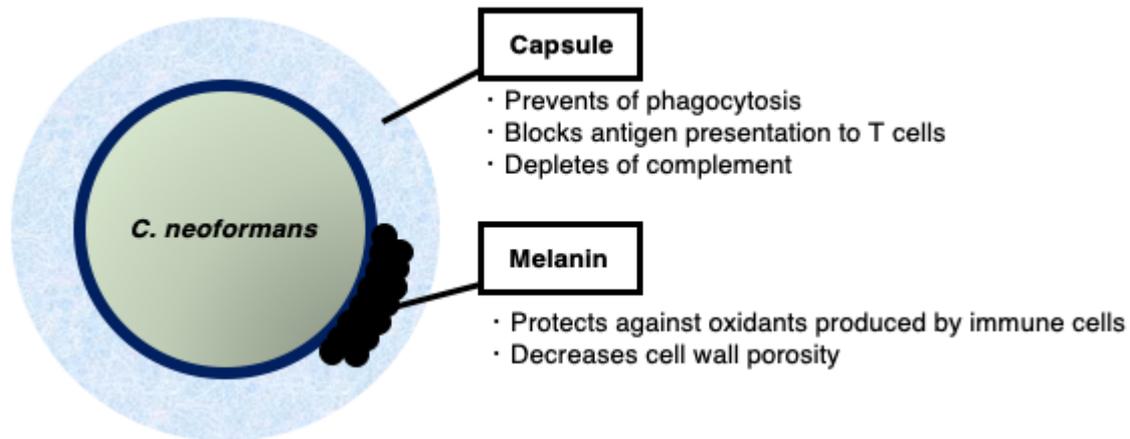


Figure 1. Functions of capsule and melanin in *C. neoformans*. *C. neoformans* produces a capsule and melanin for escaping host defense systems. The capsule contributes to preventing phagocytosis, blocking antigen presentation to T cells, and depleting complement. Melanin is involved in protecting against the oxidants produced by immune cells and decreasing cell wall porosity. *C. neoformans* cells are surrounded by a capsule layer and the cell wall is coated with melanin.

2. Signal transduction pathways related to the pathogenicity of *Cryptococcus neoformans*

C. neoformans regulates signal transduction pathways for infection in immunocompromised humans. This review focuses on the calcineurin, G-protein alpha 1 subunit (Gpa1)-cAMP, and protein kinase C 1 (Pkc1)-mitogen-activated protein kinase (MAPK) pathways, because genetic studies indicate that these three signal transduction pathways are required for the pathogenicity of *C. neoformans* (Figure 2).

2.1. Calcineurin signal transduction pathway (Figure 2A)

Calcineurin, a serine/threonine phosphatase, is present in all eukaryotes and plays an important role in the calcium-dependent signal transduction pathway (19). *C. neoformans* has a calcineurin signal transduction pathway and requires calcineurin for growth at 37°C, which is human body temperature (17,20). In *C. neoformans*, calcineurin comprises Cna1 and Cnb1. Calcium-bound calmodulin 1 (Cam1) forms a complex with Cna1 and Cnb1 by binding to the C-terminal region of Cna1 (17). The *cna1* gene-deficient *C. neoformans* mutant exhibits reduced pathogenicity in several animals, such as nematodes (*C. elegans*), wax moths (*G. mellonella*), silkworms (*Bombyx mori*), and mice (16,21-25). Therefore, the calcineurin signal transduction pathway plays an important role in the pathogenicity of *C. neoformans* by allowing *C. neoformans* to adapt to the host body temperature. Activated calcineurin complex dephosphorylates the transcription factor Crz1, which then transfers from the cytoplasm to the nucleus to regulate gene expression (26). Therefore, transcriptional regulation by Crz1 functions in the calcineurin signal transduction pathway in *C. neoformans* (26). In a *Galleria mellonella*

infection model, *crz1* gene-deficient *C. neoformans* mutants exhibit reduced pathogenicity.

The *cna1* and *crz1* gene-deficient *C. neoformans* mutants are sensitive to Congo red, an agent that induces stress to the cell wall, and the detergent sodium dodecyl sulfate, an agent that disrupts cell membranes (26). Therefore, the calcineurin signal transduction pathway regulates gene expression via the transcription factor Crz1 to protect against high temperatures, cell wall stress, and membrane damage in the host environment; and the regulatory system is required for the pathogenicity of *C. neoformans*.

2.2. Gpa1-cAMP signal transduction pathway (Figure 2B)

G-protein coupled receptors (GPCRs) respond to various stimuli, and downstream signaling molecules are regulated by G-protein activation (27). *C. neoformans* has several GPCRs on the cell membrane surface (18). Gpa1 is the alpha subunit of the G protein and is required for *C. neoformans* pathogenicity (28). Gpa1 binds to adenylyl cyclase (Cac1) and increases its enzymatic activity (17). Cac1 produces cAMP from ATP (17). The increase in the intracellular cAMP levels leads to increased cAMP binding with the protein kinase regulatory (Pkr) protein (17). Pkr binds to protein kinase A1 (Pka1), and dissociates from Pka1 by binding to cAMP (27). The dissociated Pka1 phosphorylates the transcription factors neuregulin 1 (Nrg1) and Rim101 (27). Phosphorylated Nrg1 and Rim101 regulate gene expression and promote the synthesis of melanin and the polysaccharide capsule (27). *C. neoformans* mutants deficient for *gpa1*, *pka1*, *cac1*, or *nrg1* have reduced pathogenicity in mice (28-31). On the other hand, *C. neoformans* mutants deficient for *rim101* do not exhibit increased pathogenicity in an intranasal mouse infection model (32). Therefore, Nrg1, but not Rim101, may play

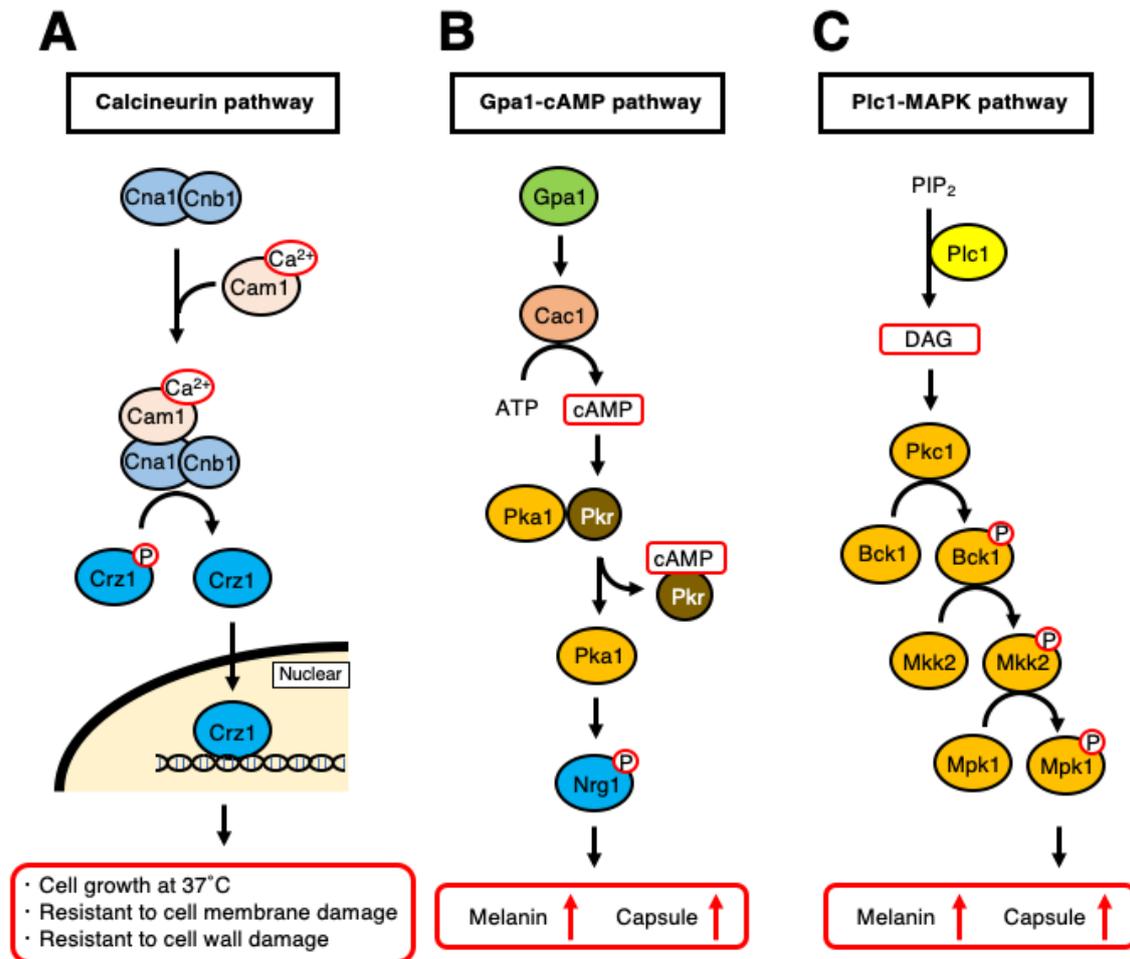


Figure 2. Signal transduction pathways related to the pathogenicity of *C. neoformans*. A. Calcineurin signal transduction pathway. Calcineurin (Cna1 and Cnb1 proteins) form a complex with calcium-bound Cam1 protein and dephosphorylates transcription factor Crz1. Dephosphorylated Crz1 regulates gene expression for resistance to high temperature, cell membrane damage, and cell wall damage. B. Gpa1-cAMP signal transduction pathway. Gpa1 protein activates Cac1 adenylyl cyclase, which produces cAMP from ATP. Intracellular cAMP binds to Pkr and releases Pka1 from the Pkr and Pka1 complex. The dissociated Pka1 phosphorylates transcription factor Nrg1, which promotes the synthesis of melanin and the polysaccharide capsule. C. Plc1-MAPK signal transduction pathway. Plc1 degrades from PIP₂ to diacylglycerol (DAG) and the produced DAG activates Pkc1 kinase, which triggers activation of the MAPK signal transduction pathway. Mpk1 is involved in the synthesis of melanin and the polysaccharide capsule.

an important role in the pathogenicity of *C. neoformans* in the Gpa1-cAMP signal transduction pathway.

2.3. Pkc1-MAPK signal transduction pathway (Figure 2C)

Phosphatidylinositol (PI) and phosphatidylinositol diphosphate (PIP₂) are lipids involved in various signal transduction pathways (17). *C. neoformans* also uses the lipid-mediated signal transduction pathway (17). PIP₂, but not PI, is degraded into diacylglycerol (DAG) by phospholipase Plc1 (33). The increase in the intracellular DAG levels leads to the phosphorylation of the MAPK kinase kinase Bck1 by the PKC Pkc1 (17,34,35). Phosphorylated Bck1 phosphorylates Mkk2, a MAPK kinase (17,34). Phosphorylated Mkk2 phosphorylates Mpk1, a MAPK (17,34). Mpk1 is a phosphorylation enzyme involved in the synthesis of

melanin and the polysaccharide capsule, and is required for the pathogenicity of *C. neoformans* (36). The *plc1* gene-deficient *C. neoformans* mutant exhibits decreased pathogenicity in invertebrate infection models (e.g., *Caenorhabditis elegans* and *Galleria mellonella*) (37,33). The *mpk1* gene-deficient *C. neoformans* mutant has reduced pathogenicity in mice infected *via* injection into the lateral tail vein (36). Therefore, the Pkc1-MAPK signal transduction pathway is essential for *C. neoformans* pathogenicity and is regulated by the intracellular levels of DAG, a lipid intermediary.

3. Environmental components related to the pathogenicity of *Cryptococcus neoformans*

Signal transduction pathways in *C. neoformans* are promoted by environmental components, including host factors (Table 1).

Table 1. Molecules that trigger signal transduction pathways related to the pathogenicity of *C. neoformans*

Signaling pathways	Calcineurin	Gpa1-cAMP	Pkc1-MAPK
Triggering molecules	Calcium Inositol	Glucose Methionine Iron Carbon dioxide	Inositol

3.1. Sugars

Glucose is a major carbon source for cells and is abundant in the human body. *C. neoformans* grows using glucose as a carbon source, and regulates the expression of virulence factors according to the glucose concentration in the environment. Glucose is a substrate for the capsule in *C. neoformans*. Moreover, glucose induces the cAMP pathway by activating Pka in *C. neoformans* (38). Therefore, factors that regulate glucose recognition and utilization may be involved in the *C. neoformans* pathogenicity. Gpr4, a receptor, and Hxt1, a hexose transporter, may regulate the recognition and utilization of glucose in *C. neoformans*. The *gpr4* gene-deficient *C. neoformans* mutant, however, does not exhibit reduced pathogenicity in mice (39). The *hxt1* gene-deficient *C. neoformans* mutant exhibits normal pathogenicity in a *C. elegans* infection model (40). Hxs1 is a high-affinity glucose transporter required for cell growth in low glucose conditions (41). The *hxs1* gene-deficient *C. neoformans* mutant exhibits reduced pathogenicity in a mouse intranasal infection model (41). Although Hxs1 contributes to melanin production, the molecular relationship between glucose transport mediated by Hxs1 and signal transduction pathways is unclear. The identification of factors that recognize or utilize glucose for modulating the pathogenicity of *C. neoformans* is an important subject for future studies.

Inositol is a sugar that many organisms can synthesize from glucose. Inositol is abundant in the human brain and is used for osmotic regulation in neuronal cells. *C. neoformans* recognizes inositol in the human brain to increase the efficiency of its blood-brain barrier penetration (42). In *C. neoformans*, inositol is taken up into cells *via* the inositol transporters ITR1a and ITR3c. *C. neoformans* mutants deficient for both *itr1a* and *itr3c* exhibit reduced pathogenicity in a mouse systemic infection model (43). Inositol activates calcineurin and MAPK signal transduction pathways after conversion to PI and PIP₂ (18). Therefore, inositol in the human brain is a host factor that enhances the pathogenicity of *C. neoformans*.

3.2. Methionine

Methionine, an amino acid, is used by all organisms for protein synthesis and abundantly exists in the human body. In *C. neoformans*, methionine activates the cAMP signal transduction pathway by increasing

the intracellular cAMP concentration (39). Gpr4 is a candidate molecule for increasing the cAMP concentration *via* the recognition of methionine. The *gpr4* gene-deficient *C. neoformans* mutant has a decreased cellular cAMP concentration and decreased capsule production (39). On the other hand, the *gpr4* gene-deficient *C. neoformans* mutant does not have reduced pathogenicity in mice (39). These findings suggest that *gpr4* contributes to the methionine response, but is not necessary for *C. neoformans* pathogenicity. Further studies are needed to investigate the gene(s) required for a response to environmental methionine and to clarify the contribution of methionine to *C. neoformans* pathogenicity using gene-deficient mutants.

3.3. Metals

Calcium is an essential metal for organisms and has various roles as an enzyme activator and signal transmitter. Microorganisms adapt to host environments *via* calcium recognition and utilization. In *C. neoformans*, calcium binds to Cam1, and the calcium-bound Cam1 forms a complex with calcineurin, leading to activation of the calcineurin signal transduction pathway (17). The *cam1* gene is required for *C. neoformans* growth (44). Therefore, calcium recognition by Cam1 is important not only for controlling pathogenicity, but also for utilizing the calcium required for survival. Moreover, the intracellular calcium concentration in *C. neoformans* is increased by inositol uptake. Activation of the calcineurin signal transduction pathway by inositol may be associated with calcium recognition *via* Cam1 (17).

Iron is required for cell growth in organisms and is abundant in human blood. *C. neoformans* alters the expression of various genes, including those involved in capsule synthesis, in response to the iron concentration in the environment (45). Iron binds to the transcription factor Cir1 and alters its transcriptional activity in *C. neoformans*. In *cir1* gene-deficient *C. neoformans* mutants, almost all of the gene expression changes caused by iron do not occur and capsule production decreases (45). Comprehensive gene expression analysis using a *cir1* gene-deficient mutant revealed that Cir1 increases the expression of genes encoding many GPCRs and the *cacl* gene, resulting in activation of the cAMP pathway (45). Furthermore, the *cir1* gene-deficient *C. neoformans* mutant exhibits

reduced pathogenicity in a mouse systemic infection model (45). Thus, iron is a host factor that regulates the *C. neoformans* pathogenicity, and Cir1 is an important factor in its recognition.

3.4. Carbon dioxide (CO₂)

CO₂ is produced by respiration and is abundant in the human body. CO₂ regulates capsule formation in *C. neoformans* (46). *C. neoformans* absorbs CO₂ and converts it to HCO₃⁻ by the β-carbonic anhydrase Can2. HCO₃⁻ binds to the adenylyl cyclase Cac1 and increases enzyme activity. Activated Cac1 increases intracellular cAMP, which leads to activation of the cAMP pathway. On the other hand, while the *can2* gene is required for growth under environmental CO₂ concentrations (0.036% CO₂), it is not required for growth at human body CO₂ concentrations (5% CO₂) (47). Moreover, the *can2* gene does not contribute to *C. neoformans* pathogenicity in mice (47). The identification of molecules that modulate pathogenicity under various CO₂ concentrations in the human body requires further study.

4. Conclusion

In this review, we described the pathogenic regulatory mechanisms of *C. neoformans*, focusing on the molecules necessary for host infection in humans. *C. neoformans* controls virulence in the host through the recognition of host factors. The contributions of several molecules to the pathogenicity of *C. neoformans* have not yet been elucidated in animal infection models. Infection models for evaluating *C. neoformans* pathogenicity have been established. The *gpa1* and *pkal* gene-deficient *C. neoformans* mutants exhibit reduced pathogenicity in invertebrate animals, such as nematodes, wax moths, and silkworms (22-24). Therefore, the importance of the Gpa1-cAMP signaling pathway for *C. neoformans* pathogenicity can be evaluated in invertebrate infection experiments. The requirement of certain factors for recognizing the host environment to control the pathogenicity of *C. neoformans* can be clarified in future studies using invertebrate infection models.

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