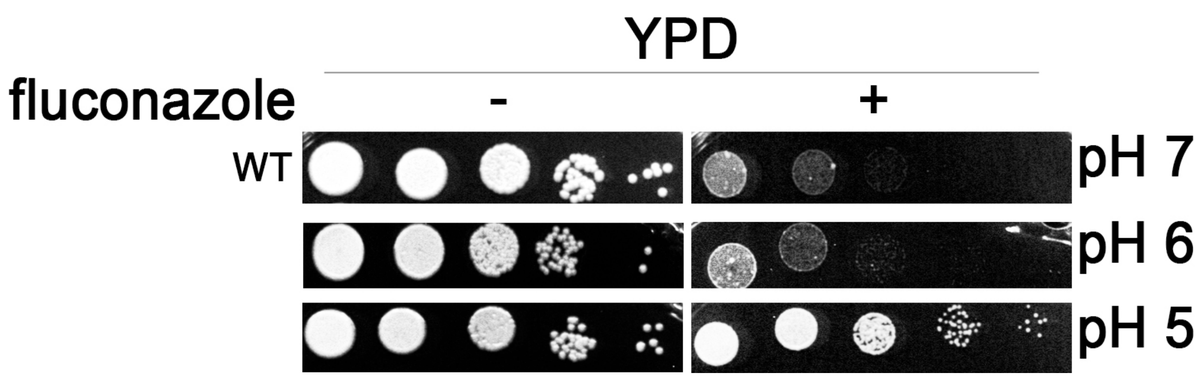
Regulation of gene expression by acidic pH in an opportunistic human fungal pathogen *Cryptococcus neoformans*: modulating antifungal susceptibility and iron Uptake

Donghyeun Kim and Won Hee Jung*

Department of Systems Biotechnology, Chung-Ang University, Anseong, 17546, Korea * whjung@cau.ac.kr

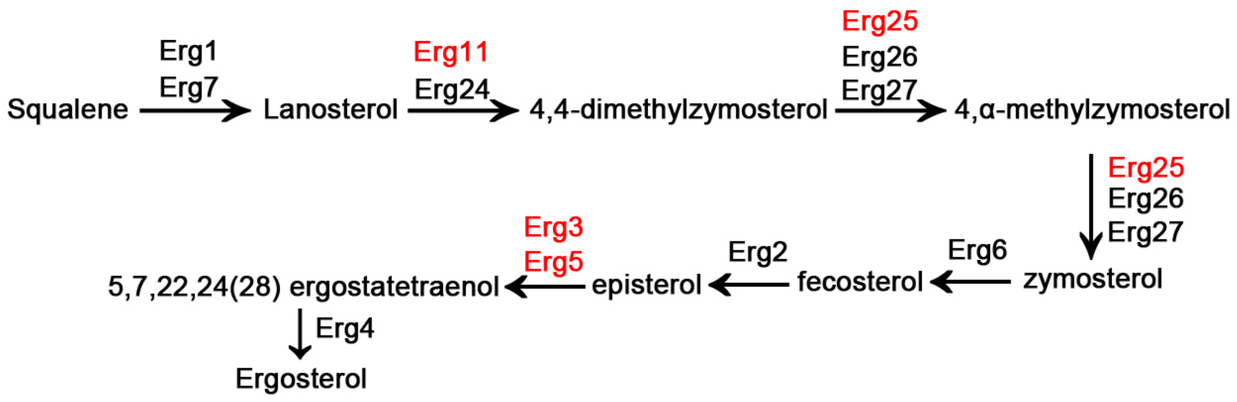
Abstract *Cryptococcus neoformans* is an opportunistic human fungal pathogen, but its infection is militated by phagocytosis and phagolysosomes in the host immune system. It has been reported that the pH of phagolysosomes containing *C. neoformans* is approximately 5.3. In general, an acidic pH condition, in comparison with neutral pH, is known to alter several physiological characteristics of fungi, including susceptibility to azole antifungal drugs. Indeed, azole antifungal susceptibility of another well know human fungal pathogen, *Candida albicans*, is modulated by pH, although the underlying mechanism is not clear yet. Therefore, we investigated whether the environmental pH influences the azole antifungal susceptibility of *C. neoformans* and how the fungus responds to acidic pH. We found that the minimal inhibitory concentration (MIC) of *C. neoformans* against fluconazole was increased under an acidic pH condition, and our GC-MS analysis revealed that the ergosterol content in *C. neoformans* that was grown under an acidic condition was greatly increased in comparison to that in cells grown at a neutral pH level. Moreover, a mutant strain lacking *CFO1*, which is the major component in the high-affinity reductive iron uptake system in *C. neoformans*, displayed significantly reduced sensitivity to fluconazole at an acidic pH level. This implies that a different iron uptake pathway governs the transport of iron under such a condition. Considering that a number of the proteins involved in ergosterol biosynthesis require iron as a cofactor, our data implied the involvement of a yet unknown iron uptake pathway, which is independent of the *CFO1* function, in azole antifungal susceptibility of *C. neoformans* under an acidic pH condition. In addition, we investigated the underlying molecular regulatory mechanism to understand how *C. neoformans* responds to an acidic pH condition through transcriptome analysis as well as phenotypic and biochemical analysis of the series mutant strains lacking the genes involved in the major iron uptake pathways.

I. Antifungal sensitivity was reduced at acidic pH.



Phenotypic analysis was carried out to investigate antifungal sensitivity of *C. neoformans*. Cells were spotted on YPD supplemented with or without 20 μg/mL of fluconazole at different pH. The sensitivity to fluconazole was reduced at acidic pH.

II. The ergosterol biosynthesis pathway in *C. neoformans*



The ergosterol biosynthesis pathway in *C. neoformans*. Red indicates the proteins required for iron as a cofactor. We hypothesized that the fungal cells grown at acidic pH import more iron than those grown at neutral pH, which causes increased expression of the ergosterol biosynthesis pathway.

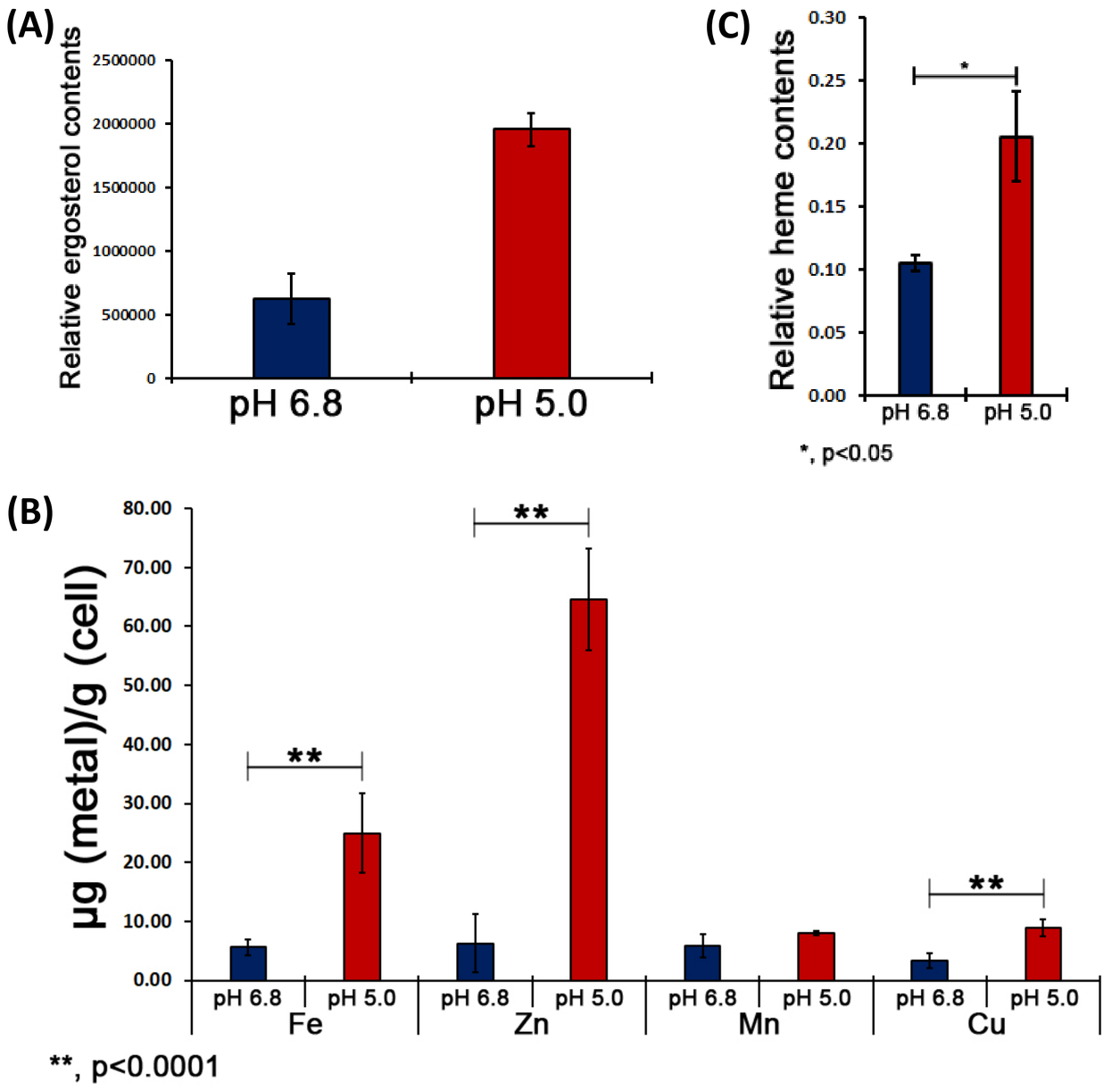
III. Transcriptome analysis revealed expression of *ERG* genes were increased at acidic pH.

Gene ID	Product	Gene	Fold change (low/high pH)
CNAG_00040	cytochrome family 51 (sterol 14-demethylase)	*ERG11*	4.80
CNAG_00117	C-14 sterol reductase	*ERG24*	1.65
CNAG_00519	lathosterol oxidase	*ERG3*	1.45
CNAG_00854	C-8 sterol isomerase	*ERG2*	1.18
CNAG_03819	sterol 24-C-methyltransferase	*ERG6*	2.41
CNAG_03009	ergosterol biosynthesis-related	*ERG28*	1.28
CNAG_02830	delta24(24(1))-sterol reductase	*ERG4*	1.98
CNAG_06829	squalene monooxygenase	*ERG1*	1.77
CNAG_07437	3-keto sterol reductase	*ERG27*	1.11
CNAG_01129	lanosterol synthase	*ERG7*	1.61
CNAG_06644	C-22 sterol desaturase	*ERG5*	2.69
CNAG_04605	sterol-4alpha-carboxylate 3-dehydrogenase (decarboxylating)	*ERG26*	1.37
CNAG_01737	methylsterol monooxygenase	*ERG25*	1.66

0.25≤ 0.5≤ 1≤ 2≤ 4≤

Transcriptome analysis was carried out to investigate expression of genes differentially expressed at acidic pH. We found that several *ERG* genes (*ERG11*, *ERG6*, and *ERG*5) were upregulated.

IV. Ergosterol, iron and heme contents were increased at acidic pH.



(A) GC-MS analysis revealed that ergosterol contents was increased at acidic pH. (B) ICP (Inductively Coupled Plasma) was performed. The metal ions including iron, zinc and copper were increased at acidic pH. (C) Heme contents were also increased at acidic pH. These results suggested that *C. neoformans* imports more iron, which is then sequentially utilized for heme and ergosterol synthesis, and influences reduced sensitivity to fluconazole at acidic pH.

Conclusions

- We showed that antifungal sensitivity of C. neoformans against fluconazole is modulated by pH.
- Our results suggested that, at acidic pH, iron uptake is increased at acidic pH, which in turn increased heme biosynthesis and ergosterol biosynthesis by upregulation of the genes involved in the pathways, and caused reduced antifungal sensitivity.

References

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