Pseudohyphal Growth in a Dimorphic Yeast, Candida maltosa, after Disruption of the C-GCN4 Gene, a Homolog of Saccharomyces cerevisiae GCN4

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The transcriptional activator protein Gcn4p increases the transcription of many genes that code for amino acid synthesis genes during amino acid starvation in Saccharomyces cerevisiae. Here we showed that after the disruption of C-GCN4, a homolog in Candida maltosa of GCN4 in S. cerevisiae, formed pseudohyphae in minimal medium. This is the first report that a GCN4 homolog is involved in the control of morphological transition.

Key words: GCN4; Candida maltosa; pseudohyphal growth; morphogenesis

Dimorphism is a peculiar characteristic of certain fungi. It is the ability to switch between yeast form and filamentous form in response to environmental conditions. Conditions that cause this transition have been studied, mainly in Candida albicans and Saccharomyces cerevisiae. Transition to the filamentous form is one of several virulence attributes that allow pathogenic yeasts to invade human tissues.1) Candida maltosa is a dimorphic fungus with a diploid genome. C. maltosa grown in a medium containing n-alkane as the sole carbon source forms pseudohyphae.2,3) A part of a centromeric DNA (CEN) region, when present on a plasmid in C. maltosa, also causes pseudohyphal growth.4) Phylogenetic analysis showed that this species is closely related to C. albicans.5) We have been studying inducible cycloheximide resistance of C. maltosa and found that this resistance was dependent on the induction of a variant ribosomal protein L41-Q. Analyses of the promoter region of a gene encoding the variant L41-Q revealed that a small region that was very similar to the Gcn4p-binding sequence of S. cerevisiae was essential for the induction of L41-Q. Thus we went to the identification of a GCN4 homolog, C-GCN4, from C. maltosa genome and prepared a mutant, namely ΔC-GCN4 in which two C-GCN4 alleles were inactivated by gene disruption technique (Takaku et al., in preparation). Gcn4p mediates the transcriptional activation of amino acid synthetic genes in S. cerevisiae by specifically binding to a DNA sequence element in their 5′-regulatory regions.6) The mutant ΔC-GCN4 grew as well as the wild-type strain in YPD medium [2% Polypepton (Wako, Osaka), 1% yeast extract, 2% glucose], but it grew more slowly and grew pseudohyphae in a minimum medium (Fig. 1A–E). To our knowledge, this is the first report of morphological change caused by mutation of GCN4 or its homologs.

The percentage of cells that had formed pseudohyphae by different times is shown in Fig. 2. Both the wild type and ΔC-GCN4 grew pseudohyphae when yeast cells were transferred from YPD to minimal medium in a nutritional downshift. In particular, the wild-type strain grew pseudohyphae transiently just after nutritional downshift only (Fig. 2, open symbols). It is possible that the same signaling pathway is activated in both strains but that C-Gcn4p suppressed pseudohyphal growth in the wild type.

At different times after a nutritional downshift, we measured the amount of C-GCN4 mRNA by northern blotting done as described previously.7,8) An 800-bp C-GCN4 fragment was prepared by PCR amplification from C. maltosa genomic DNA with primers 5′-CTGAAGCTTCTTTAGCTG-3′ and 5′-GCTTGTTTTTCAACCAAC-3′ (oligo-DNAs were synthesized by Hokkaido System Science, Sapporo). The fragment was labeled with [α-32P]dCTP with a random primer DNA labeling kit (Ver. 2, Takara). A 500-bp S. cerevisiae ACT1 hybridization probe labeled with 32P was prepared in a similar way. The signals were measured with correction by those of ACT1 mRNA. The C-GCN4 mRNA levels increased to 6-fold by 20 min after the nutritional downshift, and then declined (Fig. 3). The pattern of change suggested that C-Gcn4p is regulated at both transcription and translation during amino acid starvation (Takaku et al. in preparation). The change in the C-GCN4 mRNA level after a nutritional downshift is likely to be a counteractive response to the downshift, and may suppress pseudohyphal growth.

Rapamycin binds and inhibits Tor protein kinases which act in a nutrient-sensing signal transduction pathway. In S. cerevisiae, a sublethal concentration

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Fig. 1. Growth and Cell Morphology of *C. maltosa* Strains.

(A) *C. maltosa* wild-type strain CMT100 (his5::HIS5, ura3::URA3, ade1::ADE1) (circles) and the mutant ΔC-GCN4 (C-GCN4a::HIS5, ura3::URA3, C-GCN4b::ADE1) (triangles) were grown on minimum liquid medium (0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate (Difco), 0.5% (NH₄)₂SO₄, 2% glucose, and 2% agar). Turbidity was monitored automatically with a Biophotorecorder (model TN-172D; Advantec, Tokyo).

(B) After strain CMT100 was transferred from YPD solid medium (YPD plus 2% agar) to minimum solid medium for a nutritional downshift, it was grown on minimal solid medium at 30°C for 1 day.

(C) The mutant ΔC-GCN4 was transferred from YPD and grown on minimum solid medium at 30°C for 2 days. (D) and (E) After nutritional downshift, CMT100 or ΔC-GCN4, respectively, was grown on minimum liquid medium at 30°C for 1 day. Cells were viewed with Nomarski optics.
of rapamycin inhibits pseudohyphal differentiation\(^9\) triggered by nitrogen starvation.\(^{10}\) Rapamycin treatment also increases \(GCN4\) mRNA translation,\(^{11}\) but nitrogen starvation, which probably activates the Tor pathway, represses \(GCN4\) mRNA translation.\(^{12}\) These findings together with ours suggest that \(GCN4\) helps to prevent pseudohyphal differentiation and that one of the functions of the Tor pathway is to suppress Gcn4p production. To test these assumptions, we treated the wild type and the \(\Delta C\)-GCN4 strain with a sublethal concentration of rapamycin (100 ng/ml) at 30 min before a nutritional downshift and monitored their morphology. Rapamycin treatment prevented pseudohyphal differentiation of wild-type cells but not that of \(\Delta C\)-GCN4 cells (Fig. 2).

That is, in spite of the inhibition of Tor kinase by rapamycin, pseudohyphal formation was caused by poor nutritional conditions in the strain lacking \(C\)-GCN4. Thus, it is likely that C-Gcn4p suppresses pseudohyphal growth caused by poor nutritional conditions and that the Tor pathway leads to pseudohyphal development in part by the downward regulation of \(C\)-GCN4.

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References

