Comparative Analyses of Homocitrate Synthase Genes of Ascomycetous Yeasts

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Most ascomycetous yeasts have 2 homocitrate synthases (HCSs). Among the fungal lysine biosynthesis-related genes, only the HCS gene was duplicated in the course of evolution. It was recently reported that HCS of Saccharomyces cerevisiae has an additional function in nuclear activities involving chromatin regulation related to DNA damage repair, which is not related to lysine biosynthesis. Thus, it is possible that the bifunctionality is associated with HCS gene duplication. Phylogenetic analysis showed that duplication has occurred multiple times during evolution of the ascomycetous yeasts. It is likely that the HCS gene duplication in S. cerevisiae occurred in the course of Saccharomyces evolution. Although the nucleosome position profiles of the two S. cerevisiae HCS genes were similar in the coding regions, they were different in the promoter regions, suggesting that they are subject to different regulatory controls. S. cerevisiae has maintained HCS activity for lysine biosynthesis and has obtained bifunctionality.

1. Introduction

Organisms synthesize lysine from 2-oxoglutarate through α-aminoadipate or from aspartic acid through diaminopimelate [1]. Animals cannot synthesize lysine. Fungi synthesize lysine through diaminopimelate. Archaea and bacteria were also believed to synthesize lysine through diaminopimelate until it was reported that the extremely thermophilic bacterium Thermus thermophilus synthesizes lysine through α-aminoadipate [5–8].

During lysine biosynthesis in the budding yeast Saccharomyces cerevisiae, α-aminoadipate is synthesized from 2-oxoglutarate and acetyl-CoA by the enzymes Lys20 or Lys21 (homocitrate synthase [HCS]), Lys4 (homoaconitase), Lys12 (homoisocitrate dehydrogenase), and α-aminoadipate amionotransferase [9]. Lysine is synthesized from α-aminoadipate by the enzymes Lys2 (aminoadipate reductase), Lys5 (phosphopantetheinyl transferase which posttranslationally modifies Lys2), Lys9 (saccharopine dehydrogenase, glutamate forming), and Lys1 (saccharopine dehydrogenase, lysine forming) [1, 4].

It has been unclear why S. cerevisiae has 2 HCSs (Lys20 and Lys21). For example, homocitrate is mainly synthesized through Lys21 during growth on ethanol, while under fermentative metabolism, Lys20 and Lys21 play redundant roles [11]. It was recently reported that Lys20 of S. cerevisiae functions in nuclear activities involving chromatin regulation that are distinct from its previously established role in lysine synthesis [12]. Lys20 of S. cerevisiae is linked to the DNA damage repair process via the histone acetyltransferase Esa1 and the H2A.Z histone variant [12]. Thus, it is possible that this bifunctionality is associated with HCS gene duplication.

2. Materials and Methods

2.1. Phylogenetic Analyses. I selected 71 HCSs (31 from Saccharomycotina species, 30 from Pezizomycotina species, 2 from Taphrinomycotina species, and 8 from Basidiomycota species) based on BLASTP results in the fungal genome database at NCBI (http://www.ncbi.nlm.nih.gov/projects/genome/guide/fungi/). Multiple alignments were generated with CLUSTAL W. A maximum likelihood tree was reconstructed using MEGA version 5 [10]. The WAG model was
Figure 1: Phylogenetic relationships among 71 fungal homocitrate synthases. The phylogenetic tree was constructed based on multiple alignment with complete deletion of gap sites using the maximum likelihood method of MEGA software [10] with 100 bootstrap analyses. The WAG model was used as the amino acid substitution model. A total of 103 amino acid sites were considered. The γ-distributed rate was considered, and the number of discrete gamma categories was 3. The gamma was 0.81; the discrete rates were 0.14, 0.65, and 2.2.
used as the amino acid substitution model. The nearest neighbor interchange was used as the maximum likelihood heuristic method. The $\gamma$-distributed rate was considered, and the number of discrete gamma categories was 3.

2.2. Nucleosome Position Comparison. Nucleosome positioning was used to compare gene promoter regions. I used nucleosome position data from S. cerevisiae BY4741 [13]. The nucleosome position profiles were compared between the promoter (1000 bases upstream of the translational start site) and coding regions (between the translational start and end site) of the HCS genes, according to a previously described method [14]. Arrows indicate the coding region.

3. Results and Discussion

The HCS phylogenetic tree (Figure 1) indicates that the HCS gene has been duplicated multiple times in the course of ascomycete evolution. The 31 HCSs of the Saccharomycotina species (ascomycetous yeasts) are encoded in 17 organisms. In contrast, the 30 HCSs of the Pezizomycotina species (filamentous ascomycetes) are encoded in 28 organisms. Thus, the number of discrete gamma categories was 3.

In addition, the phylogenetic analysis based on HCS amino acid sequences, I compared the nucleosome positioning of LYS20 and LYS21. Interestingly, nucleosomes were mapped to the HCS gene promoters more often than to the coding regions (Figure 2). Nucleosome position profiles in the coding regions were highly correlated (Spearman's rank correlation coefficient = 0.833) between LYS20 and LYS21. On the other hand, those in the gene promoter regions were poorly correlated (Spearman's rank correlation coefficient = 0.396). This result suggests that these 2 HCS genes have different regulatory systems.

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References

[1] H. Xu, B. Andi, J. Qian, A. H. West, and P. F. Cook, “The α-aminoacid pathway for lysine biosynthesis in fungi,” Cell Biochemistry and Biophysics, vol. 46, no. 1, pp. 43–64, 2006.
[2] H. P. Broquist, “Lysine biosynthesis (yeast),” Methods in Enzymology, vol. 17, no. 2, pp. 112–113, 1971.
[3] H. J. Vogel, “Distribution of lysine pathways among fungi: evolutionary implications,” American Naturalist, vol. 98, pp. 435–446, 1964.
[4] T. M. Zabriskie and M. D. Jackson, “Lysine biosynthesis and metabolism in fungi,” *Natural Product Reports*, vol. 17, no. 1, pp. 85–97, 2000.

[5] N. Kobashi, M. Nishiyama, and M. Tanokura, “Aspartate kinase-independent lysine synthesis in an extremely thermophilic bacterium, *Thermus thermophilus* lysine is synthesized via α-amino adipic acid not via diaminopimelic acid,” *Journal of Bacteriology*, vol. 181, no. 6, pp. 1713–1718, 1999.

[6] T. Kosuge and T. Hoshino, “Lysine is synthesized through the α-amino adipate pathway in *Thermus thermophilus*,” *FEMS Microbiology Letters*, vol. 169, no. 2, pp. 361–367, 1998.

[7] H. Nishida, M. Nishiyama, N. Kobashi, T. Kosuge, T. Hoshino, and H. Yamane, “A prokaryotic gene cluster involved in synthesis of lysine through the amino adipate pathway: a key to the evolution of amino acid biosynthesis,” *Genome Research*, vol. 9, no. 12, pp. 1175–1183, 1999.

[8] H. Nishida, “Distribution of genes for lysine biosynthesis through the amino adipate pathway among prokaryotic genomes,” *Bioinformatics*, vol. 17, no. 2, pp. 189–191, 2001.

[9] I. Iraqui, S. Vissers, M. Cartiaux, and A. Urrestarazu, “Characterisation of *Saccharomyces cerevisiae* ARO8 and ARO9 genes encoding aromatic aminotransferases I and II reveals a new aminotransferase subfamily,” *Molecular and General Genetics*, vol. 257, no. 2, pp. 238–248, 1998.

[10] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar, “MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods,” *Molecular Biology and Evolution*, vol. 28, no. 10, pp. 2731–2739, 2011.

[11] H. Quezada, C. Aranda, A. DeLuna et al., “Specialization of the paralogue *LYS21* determines lysine biosynthesis under respiratory metabolism in *Saccharomyces cerevisiae*,” *Microbiology*, vol. 154, no. 6, pp. 1656–1667, 2008.

[12] E. M. Scott and L. Pillus, “Homocitrate synthase connects amino acid metabolism to chromatin functions through Esa1 and DNA damage,” *Genes and Development*, vol. 24, no. 17, pp. 1903–1913, 2010.

[13] T. Matsumoto, C.-S. Yun, H. Yoshikawa, and H. Nishida, “Comparative studies of genome-wide maps of nucleosomes between deletion mutants of *elp3* and *hos2* genes of *Saccharomyces cerevisiae*,” *Plos ONE*, vol. 6, no. 1, Article ID e16372, 2011.

[14] H. Nishida, “Calculation of the ratio of the mononucleosome mapping number to the dinucleosome mapping number for each nucleotide position in the *Aspergillus fumigatus* genome,” *Open Access Bioinformatics*, vol. 1, pp. 1–6, 2009.

[15] H. Nishida and M. Nishiyama, “What is characteristic of fungal lysine synthesis through the α-amino adipate pathway?” *Journal of Molecular Evolution*, vol. 51, no. 3, pp. 299–302, 2000.

[16] M. Kellis, B. W. Birren, and E. S. Lander, “Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*,” *Nature*, vol. 428, no. 6983, pp. 617–624, 2004.

[17] R. T. Morris and G. Drouin, “Ectopic gene conversions in the genome of ten hemiascomycete yeast species,” *International Journal of Evolutionary Biology*, 2011, Article ID 970768, 11 pages, 2011.

[18] K. H. Wolfe and D. C. Shields, “Molecular evidence for an ancient duplication of the entire yeast genome,” *Nature*, vol. 387, no. 6634, pp. 708–713, 1997.

[19] M. A. Hibbs, D. C. Hess, C. L. Myers, C. Huttenhower, K. Li, and O. G. Troyanskaya, “Exploring the functional landscape of