Research Article

Evolutionary Conservation Levels of Subunits of Histone-Modifying Protein Complexes in Fungi

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Received 20 August 2008; Revised 17 November 2008; Accepted 17 February 2009

1. Introduction

Chromatin is the most important structure for the maintenance of the eukaryotic genomic DNA. The eukaryotic genomic DNA is packaged with histone proteins to form nucleosomes (the fundamental repeating unit of chromatin). Chromatin structure depends on the modification of nucleosome core histones [1, 2]. Generally the proteins that are evolutionarily conserved and distributed among a wide range of organisms play an important role in the biological processes [3]. Eukaryotes possess a variety of histone-modifying protein complexes [4, 5]. Some subunits of histone-modifying protein complexes have been evolutionarily conserved among eukaryotes, while some subunits are evolutionary lineage-specific. For example, the histone-modifying protein Ccr4 methylates histone H3 at lysine 9, which plays a major role in RNA-mediated heterochromatin formation in the fission yeast S. pombe [5]. However, the budding yeast Saccharomyces cerevisiae lacks Ccr4 homologue [6]. There has been considerable progress in the studies on fungal histone modifications in S. cerevisiae and S. pombe. Most gene functions in other fungi have been annotated based on the structural similarity of their genes with the genes of the abovementioned 2 yeasts whose functions have been studied well (inferred from the results of biological experiments). In this study, I used the subunits of histone-modifying protein complexes extracted from Saccharomyces and Schizosaccharomyces. The purpose of this study is to show the evolutionary conservation levels of the subunits of fungal histone-modifying protein complexes.

2. Materials and Methods

The sequences of coding for subunits of histone-modifying protein complexes in Saccharomyces and Schizosaccharomyces were extracted from 2 major genome databases, Saccharomyces Genome Database (http://www.yeastgenome.org/), and S. pombe GeneDB (http://www.genedb.org/genedb/pombe/) at the Wellcome Trust Sanger Institute. In order to identify proteins homologous to the extracted Saccharomyces and Schizosaccharomyces proteins, a BLASTP search was performed for 9 complete fungal genomes (6 ascomycetes species, namely, Aspergillus...
Table 1: Evolutionary conservation levels of *Saccharomyces* proteins related to histone modifications.

| Protein | Function | Score based on E value in BLASTP |
|---------|----------|----------------------------------|
| ELP3    | HAT catalytic | kla yli spo ncr afrm cne uma ncu Total |
| RPD3    | HDAC catalytic | 5 5 5 5 5 5 5 5 5 40 |
| TRA1    | HDAC catalytic | 5 5 5 5 5 5 5 2 2 38 |
| ESA1    | HAT associated | 5 5 4 4 4 4 4 4 4 33 |
| GCN5    | HAT catalytic | 5 5 4 4 4 4 4 3 3 33 |
| HDA1    | HDAC catalytic | 5 5 4 3 4 4 4 2 2 32 |
| TAF1    | HAT catalytic | 5 5 4 3 3 3 3 3 3 31 |
| SIN3    | HDAC associated | 5 4 3 5 4 3 4 2 30 |
| SET2    | HMT catalytic | 5 3 4 2 4 3 3 2 26 |
| HST1    | HDAC catalytic | 5 3 3 3 3 3 3 2 25 |
| NTO1    | HAT catalytic | 5 3 3 3 3 3 3 3 2 25 |
| SAS3    | HAT catalytic | 4 3 3 3 3 3 3 3 3 25 |
| HAT2    | HAT associated | 4 3 3 3 3 3 3 3 3 24 |
| HSF3    | HDAC catalytic | 5 2 2 2 2 2 2 2 2 24 |
| RPH1    | HDMT catalytic | 4 4 2 3 3 3 3 3 3 24 |
| SIR2    | HDAC catalytic | 4 3 3 3 3 3 3 3 3 24 |
| JHD2    | HDMT catalytic | 5 3 3 3 3 3 3 3 3 24 |
| HST2    | HDAC catalytic | 5 2 3 2 3 3 3 3 3 22 |
| SET1    | HMT catalytic | 5 3 2 3 3 3 3 2 2 22 |
| SWD1    | HMT associated | 5 3 3 3 3 3 3 2 2 21 |
| EAF1    | HAT associated | 5 3 2 2 2 2 2 2 2 20 |
| EPL1    | HAT associated | 5 3 2 2 3 3 3 2 2 20 |
| SWD2    | HMT associated | 5 2 3 2 3 2 2 2 2 20 |
| HAT1    | HAT catalytic | 4 3 3 2 2 3 2 2 2 19 |
| SAS2    | HAT catalytic | 4 3 2 2 2 2 2 2 2 19 |
| SET3    | HDAC associated | 4 3 2 2 2 2 2 2 2 19 |
| SIF2    | HDAC associated | 4 3 2 2 2 2 2 2 2 19 |
| GIS1    | HDMT catalytic | 3 3 2 2 2 2 2 2 2 19 |
| HDA3    | HDAC catalytic | 5 3 1 2 2 1 2 2 2 18 |
| HOS1    | HAT catalytic | 5 3 2 2 2 2 3 2 2 18 |
| JHD1    | HDMT catalytic | 4 3 2 2 3 2 2 2 2 18 |
| SPT10   | HAT catalytic | 4 3 3 2 3 2 2 3 2 18 |
| SPP1    | HMT associated | 3 2 2 2 2 2 2 2 2 17 |
| SWD3    | HMT associated | 3 2 2 2 2 2 2 2 2 17 |
| YAF9    | HAT associated | 3 2 2 2 2 2 2 2 2 17 |
| YNG2    | HAT associated | 3 2 2 2 2 2 2 2 2 17 |
| BRE2    | HMT associated | 4 2 2 2 2 2 2 2 2 16 |
| NAT4    | HAT catalytic | 2 2 2 2 2 2 2 2 2 16 |
| PHO23   | HDAC catalytic | 2 2 2 2 2 2 2 2 2 16 |
| RTT109  | HAT catalytic | 4 2 2 2 2 2 2 2 2 16 |
| SAS5    | SMT catalytic | 2 2 2 2 2 2 2 2 2 16 |
| YNG1    | HAT associated | 2 2 2 2 2 2 2 2 2 16 |
| IST1    | HMT catalytic | 2 2 2 2 2 2 2 2 2 16 |
| ROQ1    | HDAC associated | 3 2 2 2 2 2 2 2 2 15 |
| SGF29   | HAT associated | 3 2 2 2 2 2 2 2 2 15 |
| SWC4    | HAT associated | 3 2 2 2 2 2 2 2 2 15 |
| HPA2    | HDAC catalytic | 2 2 2 2 2 2 2 2 2 14 |
| RXT3    | HDAC associated | 2 2 2 2 2 2 2 2 2 14 |
| SGF73   | HAT associated | 2 2 2 2 2 2 2 2 2 14 |
| EAF7    | HAT associated | 2 2 2 2 2 2 2 2 2 12 |
| RXT2    | HDAC associated | 3 2 2 2 2 2 2 2 2 11 |
| SDC1    | HMT associated | 2 2 2 2 2 2 2 2 2 11 |
| SGF11   | HAT associated | 2 2 2 2 2 2 2 2 2 11 |
| AHC1    | HAT associated | 3 2 0 2 2 2 2 2 2 9 |
| EAF6    | HAT catalytic | 2 2 1 2 2 2 2 2 2 9 |
| HDA2    | HDAC catalytic | 3 2 1 1 1 1 1 1 1 9 |
| HIF1    | HAT associated | 2 2 1 2 2 2 2 2 2 8 |
| SAP30   | HDAC catalytic | 2 2 1 2 2 2 2 2 2 8 |
| SAS4    | HAT catalytic | 2 2 1 2 2 2 2 2 2 8 |
| SHG1    | HAT associated | 2 2 2 0 1 0 0 0 0 7 |
| RFM1    | HDAC associated | 2 0 0 0 1 0 0 0 0 3 |

HAT, histone acetyltransferase; HDAC, histone deacetylase; HDMT, histone demethylase; HMT, histone methyltransferase

asm, Aspergillus fumigatus; eeu, Encephalitozoon cuniculi; cne, Cryptococcus neoformans; kla, Kluyveromyces lactis; ncr, Neurospora crassa; spo, Schizosaccharomyces pombe; uma, Ustilago maydis; yli, Yarrowia lipolytica.
fumigatus, Kluyveromyces lactis, Neurospora crassa, S. cerevisiae, S. pombe, and Yarrowia lipolytica; 2 basidiomycetes species, namely, Cryptococcus neoformans, Ustilago maydis, and 1 microsporidium, namely, Encephalitozoon cuniculi) in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [7]. Based on the E values of the BLASTP search results, I classified 6 evolutionary conservation levels and scored them as follows: score 0, not detected; score 1, $E$ value $> 10^{0}$; score 2, $10^{-50} < E$ value $\leq 10^{-3}$; score 3, $10^{-100} < E$ value $\leq 10^{-50}$; score 4, $10^{-150} < E$ value $\leq 10^{-100}$; score 5, $E$ value $\leq 10^{-150}$. Based on the total of their scores, the subunits were ranked.

The Molecular Evolutionary Genetics Analysis (MEGA) software [8] was used to generate a neighbor-joining tree with 1000 bootstrap replicates from multiple alignments with all the gap sites deleted. A total of 496 amino acid sites were considered. PHYLIP software [9] was used to generate a neighbor-joining tree with 100 bootstrap replicates. The JTT model was used as the model of amino acid substitution.

Number of times to jumble in the PROML program was 2.

### 3. Results and Discussion

From the 2-yeast genome databases, I extracted 62 and 48 subunits of histone-modifying protein complexes of S. cerevisiae and S. pombe, respectively. Among the 110 subunits, 34 Saccharomyces and 24 Schizosaccharomyces proteins were catalytic subunits; the others were associated complex subunits. The evolutionary conservation levels of the 62 Saccharomyces and 48 Schizosaccharomyces proteins are shown in Tables 1 and 2, respectively.

The histone acetyltransferase (HAT) catalytic subunit ELP3 was found to be the most conserved among the 62 Saccharomyces and 48 Schizosaccharomyces subunits. The main acetylation sites of ELP3 are lysine-14 of histone H3 and lysine-8 of histone H4 [10]. In addition, ELP3 is an integral subunit of elongating RNA polymerase II holoenzyme in S. cerevisiae, which is involved in transcription-associated chromatin modification and remodeling [11, 12]. The deletion of ELP3 gene in yeast confers slow growth adaptation, slow gene activation, and temperature sensitivity.

![Phylogenetic relationships among the histone acetyltransferase catalytic subunit ELP3 and its homologues.](image)
| Protein        | Function                        | Score based on E value in BLASTP | Total |
|---------------|---------------------------------|-----------------------------------|-------|
| ELP3          | HAT catalytic                   | 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 40 |       |
| CLR6          | HDAC catalytic                  | 5 5 5 5 5 5 5 5 5 5 5 4 4 4 3 39 |       |
| HDAC2         | HDAC catalytic                  | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 2 34 |       |
| TRA1          | HAT associated                  | 5 5 5 5 5 5 5 5 5 2 2 2 2 2 2 2 34 |       |
| CLR3          | HDAC catalytic                  | 4 4 4 4 4 4 4 4 4 4 4 2 2 2 2 32 |       |
| GCN5          | HAT catalytic                   | 4 4 4 4 4 4 4 4 4 4 4 3 3 3 3 31 |       |
| MST1          | HAT catalytic                   | 4 4 4 4 4 4 4 4 4 4 4 3 3 3 3 31 |       |
| SET2          | HMT catalytic                   | 4 4 4 4 4 4 4 4 4 4 4 3 3 3 3 29 |       |
| PST1          | HDAC associated                 | 3 3 4 4 4 4 3 3 3 3 3 3 3 3 3 26 |       |
| MST2          | HAT catalytic                   | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 25 |       |
| FKB1          | HDAC associated                 | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 24 |       |
| IM2           | HDMT catalytic                  | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 23 |       |
| SET1          | HMT catalytic                   | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 23 |       |
| HST2          | HDAC catalytic                  | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 22 |       |
| NTO1          | HAT associated                  | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 22 |       |
| PST2          | HDAC associated                 | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 22 |       |
| SIR2          | HDAC catalytic                  | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 2 22 |       |
| SWD1          | HMT associated                  | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 2 22 |       |
| HST1          | HDMT catalytic                  | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 2 21 |       |
| SPAC25H1.06   | HAT associated                  | 2 2 2 3 3 3 3 3 3 3 3 3 3 3 2 20 |       |
| SWD2          | HMT associated                  | 2 2 3 3 2 3 2 3 2 2 2 2 2 2 2 18 |       |
| SWD3          | HMT associated                  | 2 2 3 2 3 2 3 2 2 2 2 2 2 2 2 18 |       |
| CLR4          | HAT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 16 |       |
| EPL1          | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 16 |       |
| RL10.09       | HAT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 16 |       |
| SET3          | HMT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 16 |       |
| SPAC17G8.07   | HAT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 16 |       |
| SPCC1235.09   | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 16 |       |
| CTI6          | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 15 |       |
| HAT1          | HAT catalytic                   | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 15 |       |
| SPAC22E12.19  | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 15 |       |
| ALP13         | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| CPH1          | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| CPH2          | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| LSD1          | HDMT catalytic                  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| LSD2          | HDMT catalytic                  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SET5          | HMT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SET6          | HMT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SET9          | HMT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SPCC1795.08c  | HAT associated                  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SPP1          | HMT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SWC4          | HAT associated                  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SET7          | HMT catalytic                   | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 14 |       |
| SPAC6F6.09    | HAT associated                  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 13 |       |
| EAF7          | HAT associated                  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 11 |       |
| SPBC428.06c   | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 8  |       |
| SDN1          | HDAC associated                 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 7  |       |
| SPCC126.13c   | HDAC associated                 | 2 1 1 1 0 1 1 1 1 1 1 1 1 1 1 7  |       |

HAT, histone acetyltransferase; HDAC, histone deacetylase; HDMT, histone demethylase; HMT, histone methyltransferase.

afm, Aspergillus fumigatus; ecu, Encephalitozoon cuniculi; cne, Cryptococcus neoformans; kla, Kluyveromyces lactis; ncr, Neurospora crassa; sce, Saccharomyces cerevisiae; uma, Ustilago maydis; yli, Yarrowia lipolytica

[11]. The ELP3 protein's function may be so important for fungi (eukaryotes) that it is the most conserved.

The phylogenetic tree based on ELP3 and its homologues show that these proteins are present across eukaryotes (Figures 1(a) and 1(b)). The phylogenetic relationships among fungal ELP3 and its homologues are consistent with the fungal classification [13]. Interestingly, the microsporidium *E. cuniculi* is not included in the fungal lineage in the neighbor-joining tree (Figure 1(a)), but it is included in the maximum likelihood tree with 44% bootstrap support (Figure 1(b)).

There are some lineage-specific subunits of histone-modifying protein complexes. For example, homologues of Dot1, the histone methyltransferase (HMT) catalytic subunit of *Saccharomyces* are present in *K. lactis* and *Y. lipolytica*—2 ascomycetous yeasts (Table 1). Dot1 methylates the histone H3 at lysine-79, which is related to gene silencing in *S. cerevisiae* [14]. This modification system is also found in
mammals [14]. However, S. pombe has no homologue of Dot1 (Table 1).

The evolutionary conservation levels of the HAT and histone deacetylase (HDAC) catalytic subunits are higher than those of the associated subunits, respectively, in Saccharomyces and Schizosaccharomyces (Figure 2). However, the conservation levels of the HMT catalytic subunits are similar to those of the associated subunits, especially in Schizosaccharomyces (Figure 2). In order to elucidate the difference in evolutionary conservation levels, I analyzed the combined data of the conservation scores of the HAT catalytic and the associated subunits, HDAC catalytic and the associated subunits, HMT catalytic and the associated subunits, and the histone demethylase (HDMT) catalytic subunits of Saccharomyces and Schizosaccharomyces. Distributions of the conservation levels of the combined data are shown in Figure 3. The P values obtained by the Wilcoxon rank-sum test for the difference between the conservation levels of the HAT catalytic and the associated subunits, HDAC catalytic and the associated subunits, and HMT catalytic and the associated subunits were .043 (<.05), .0027 (<.05), and .90 (>0.05), respectively. Thus, the null hypothesis (conservation levels of catalytic and the associated subunits are equal) was rejected in the case of the histone acetylation and deacetylation systems, but not for the histone methylation system.

The results of this study show that histone acetylase and deacetylase catalytic subunits are more conserved than other subunits (Figures 2 and 3). In fact, out of the 10 most abundant proteins of Saccharomyces and Schizosaccharomyces, 9 were histone acetylation or deacetylation related proteins, that is, 4 HAT catalytic, 3 HDAC catalytic, 1 HAT associated, 1 HDAC associated, and 1 HMT catalytic subunits.
In fungal histone acetylation and deacetylation, the catalytic subunits of protein complexes are conserved and the associated subunits are evolutionary lineage-specific. However, in fungal histone methylation, both the catalytic and the associated subunits are evolutionary lineage-specific. Although the histone modification systems work cooperatively, these results strongly suggest that the evolution of the fungal histone acetylation/deacetylation system was different from that of the histone methylation system.

Acknowledgment

This study was supported by a Grant from the Institute for Fermentation, Osaka.

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