Research Article

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

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Eukaryotes possess a variety of histone-modifying protein complexes. Generally, a histone-modifying protein complex consists of multiple subunits, that is, a catalytic subunit and the associated subunits. In this study, I analyzed 62 and 48 subunits of the histone-modifying protein complexes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively. The evolutionary conservation levels of the 110 subunits were measured. The measurements revealed that the conservation levels of the catalytic subunits are significantly higher than those of the associated subunits of the histone acetyltransferase and deacetylase complexes; however, the conservation level of the catalytic subunits is similar to that of the associated subunits of the histone methyltransferase complexes. Thus, in the fungal histone acetylation and deacetylation systems, the catalytic subunits of histone-modifying protein complexes are conserved and the associated subunits are evolutionary lineage-specific. In contrast, in the fungal histone methylation system, both the catalytic and the associated subunits are evolutionary lineage-specific.

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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 Clr4 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 Clr4 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 <i>E</i> value in BLASTP								Total
		kla	yli	spo	ncr	afm	cne	uma	ecu	
ELP3	HAT catalytic	5	5	5	5	5	5	5	5	40
RPD3	HDAC catalytic	5	5	4	5	5	5	5	4	38
HOS2	HDAC catalytic	5	5	4	4	4	4	4	4	34
TRA1	HAT associated	5	5	5	5	5	5	2	2	34
ESA1	HAT catalytic	5	5	4	4	4	4	4	3	33
GCN5	HAT catalytic	5	5	4	4	4	4	4	3	33
HDA1	HDAC catalytic	5	5	5	4	3	4	4	2	32
TAF1	HAT catalytic	5	5	4	3	4	3	4	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	2	25
SAS3	HAT catalytic	4	3	3	3	3	3	3	3	25
HAT2	HAT associated	4	3	3	3	3	3	3	2	24
HOS3	HDAC catalytic	5	4	2	3	3	2	3	2	24
RPH1	HDMT catalytic	4	4	2	3	3	3	3	2	24
SIR2	HDAC catalytic	4	3	3	3	3	3	3	2	24
JHD2	HDMT catalytic	5	3	3	3	3	3	3	0	23
HST2	HDAC catalytic	3	2	3	3	3	3	3	2	22
SET1	HMT catalytic	5	3	2	3	3	2	2	2	22
SWD1	HMT associated	4	3	3	3	2	2	2	2	21
EAF1	HAT associated	5	3	2	2	2	2	2	2	20
EPL1	HAT associated	4	3	2	2	3	2	2	2	20
SWD2	HMT associated	4	3	2	3	2	2	2	2	20
HAT1	HAT catalytic	4	3	3	2	2	3	2	0	19
SAS2	HAT catalytic	4	3	2	2	2	2	2	2	19
SET3	HDAC associated	4	3	2	2	2	2	2	2	19
SIF2	HDAC associated	4	3	2	2	2	2	2	2	19
GIS1	HDMT catalytic	3	3	2	2	2	2	2	2	18
HDA3	HDAC catalytic	5	3	1	2	2	1	2	2	18
HOS1	HDAC catalytic	4	2	2	2	2	2	2	2	18
JHD1	HDMT catalytic	4	3	2	2	3	2	2	0	18
SPT10	HAT catalytic	4	3	2	2	3	2	2	0	18
SPP1	HMT associated	3	2	2	2	2	2	2	2	17
SWD3	HMT associated	3	2	2	2	2	2	2	2	17
YAF9	HAT associated	3	2	2	2	2	2	2	2	17
YNG2	HAT associated	3	2	2	2	2	2	2	2	17
BRE2	HMT associated	4	2	2	2	2	2	2	0	16
NAT4	HAT catalytic	2	2	2	2	2	2	2	2	16
PHO23	HDAC catalytic	2	2	2	2	2	2	2	2	16
RTT109	HAT catalytic	4	2	2	2	2	2	2	0	16
SAS5	HAT catalytic	2	2	2	2	2	2	2	2	16
YNG1	HAT associated	2	2	2	2	2	2	2	2	16
DOT1	HMT catalytic	4	3	0	2	2	2	2	0	15
RCO1	HDAC associated	3	2	2	2	2	2	2	0	15
SGF29	HAT associated	3	2	2	2	2	2	2	0	15
SWC4	HAT associated	3	2	2	2	2	2	2	0	15
HPA2	HAT catalytic	2	2	2	2	2	2	2	0	14
RXT3	HDAC associated	2	2	2	2	2	2	2	0	14
SGF73	HAT associated	2	2	2	2	2	2	2	0	14
EAF7	HAT associated	2	2	2	2	0	2	2	0	12
RXT2	HDAC associated	3	2	2	2	2	0	0	0	11
SDC1	HMT associated	2	2	2	2	2	0	0	1	11
SGF11	HAT associated	2	2	2	0	2	1	2	0	11
AHC1	HAT associated	3	2	0	2	2	0	0	0	9
EAF6	HAT catalytic	2	2	1	2	2	0	0	0	9
HDA2	HDAC catalytic	3	2	1	1	1	1	0	0	9
HIF1	HAT associated	2	0	1	2	1	2	0	0	8
SAP30	HDAC catalytic	2	2	1	1	2	0	0	0	8
SAS4	HAT catalytic	2	2	1	2	1	0	0	0	8
SHG1	HMT associated	2	2	2	0	1	0	0	0	7
RFM1	HDAC associated	2	0	0	0	1	0	0	0	3

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*; spo, *Schizosaccharomyces pombe*; uma,*Ustilago maydis*; yli, *Yarrowia lipolytica*.

FIGURE 1: Phylogenetic relationships among the histone acetyltransferase catalytic subunit ELP3 and its homologues. A total of 496 amino acid sites were considered from multiple alignments with all the gap sites deleted. (a) Neighbor-joining tree was generated with 1000 bootstrap replicates using the MEGA software [8]. The number at each node represents the percentage obtained in the bootstrap analysis. The bar indicates a 5% difference in the evolutionary distance. (b) Maximum likelihood tree was generated with 100 bootstrap replicates using the PHYLIP software [9]. The number at each node represents the percentage obtained in the bootstrap analysis. The JTT model was used as the model of amino acid substitution. Number of times to jumble in the PROML program was 2.

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, $10^{-50} < E \text{ value} \leq 10^0$; score 3, $10^{-100} < E \text{ value} \leq 10^{-50}$; score 4, $10^{-150} < E \text{ value} \leq 10^{-100}$; 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 maximum likelihood 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

TABLE 2: Evolutionary conservation levels of *Schizosaccharomyces* proteins related to histone modifications.

Protein	Function	Score based on <i>E</i> value in BLASTP								Total
		sce	kla	yli	ncr	afm	cne	uma	ecu	
ELP3	HAT catalytic	5	5	5	5	5	5	5	5	40
CLR6	HDAC catalytic	5	5	5	5	5	5	5	4	39
HOS2	HDAC catalytic	4	4	5	4	4	5	4	4	34
TRA1	HAT associated	5	5	5	5	5	5	2	2	34
CLR3	HDAC catalytic	4	5	5	4	4	4	4	2	32
GCN5	HAT catalytic	4	4	4	4	4	4	4	3	31
MST1	HAT catalytic	4	4	4	4	4	4	4	3	31
SET2	HMT catalytic	4	4	4	4	4	4	3	2	29
PST1	HDAC associated	3	3	4	4	4	3	3	2	26
MST2	HAT catalytic	3	3	3	3	4	3	3	3	25
PRW1	HDAC associated	3	3	3	3	3	3	3	3	24
JMJ2	HDMT catalytic	3	3	3	3	3	3	3	2	23
SET1	HMT catalytic	3	3	3	3	3	3	3	2	23
HST2	HDAC catalytic	3	3	2	3	3	3	3	2	22
NTO1	HAT associated	3	3	2	3	3	3	3	2	22
PST2	HDAC associated	3	3	3	2	3	3	3	2	22
SIR2	HDAC catalytic	3	3	2	3	3	3	3	2	22
SWD1	HMT associated	3	3	3	3	3	3	2	2	22
HST4	HDAC catalytic	3	3	3	3	3	2	2	2	21
SPAC25H1.06	HAT associated	2	2	2	3	3	3	3	2	20
SWD2	HMT associated	2	2	3	2	3	2	2	2	18
SWD3	HMT associated	2	2	3	2	3	2	2	2	18
CLR4	HMT catalytic	2	2	2	2	2	2	2	2	16
EPL1	HAT associated	2	2	2	2	2	2	2	2	16
RTT109	HAT catalytic	2	2	2	2	2	2	2	2	16
SET3	HMT catalytic	2	2	2	2	2	2	2	2	16
SPAC17G8.07	HAT catalytic	2	2	2	2	2	2	2	2	16
SPCC1235.09	HDAC associated	2	2	2	2	2	2	2	2	16
CTI6	HDAC associated	2	2	2	2	2	2	2	1	15
HAT1	HAT catalytic	3	2	2	2	2	2	2	0	15
SPAC22E12.19	HDAC associated	2	2	2	2	2	2	2	1	15
ALP13	HDAC associated	2	2	2	2	2	2	2	0	14
CPH1	HDAC associated	2	2	2	2	2	2	2	0	14
CPH2	HDAC associated	2	2	2	2	2	2	2	0	14
LSD1	HDMT catalytic	2	2	2	2	2	2	2	0	14
LSD2	HDMT catalytic	1	2	2	2	2	2	2	1	14
SET5	HMT catalytic	2	2	2	2	2	2	2	0	14
SET6	HMT catalytic	2	2	2	2	2	2	2	0	14
SET9	HMT catalytic	2	2	2	2	2	2	2	0	14
SPCC1795.08c	HAT associated	2	2	2	2	2	2	2	0	14
SPP1	HMT associated	2	2	2	2	2	2	2	0	14
SWC4	HAT associated	2	2	2	2	2	2	2	0	14
SET7	HMT catalytic	2	2	2	2	1	2	2	0	13
SPAC6F6.09	HAT associated	2	2	2	2	2	1	2	0	13
EAF7	HAT associated	2	2	2	2	0	2	0	1	11
SPBC428.06c	HDAC associated	1	1	2	2	2	0	0	0	8
SDS3	HDAC associated	0	2	2	0	2	0	1	0	7
SPCC126.13c	HDAC associated	2	1	1	0	1	1	1	0	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

FIGURE 2: Boxplots of evolutionary conservation levels of subunits of histone-modifying protein complexes. 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 $\leq 10^0$; score 3, $10^{-100} < E$ value $\leq 10^{-50}$; score 4, $10^{-150} < E$ value $\leq 10^{-100}$; score 5, E value $\leq 10^{-150}$. Each circle indicates the total of the scores of each protein. Top and bottom boxplots are based on the *Saccharomyces* and *Schizosaccharomyces* proteins, respectively. Boxes are composed of medians with first and third quartiles from the scores of the subunits. HATc, histone acetyltransferase (HAT) catalytic subunits; HATa, HAT-associated subunits; HDACc, histone deacetylase (HDAC) catalytic subunits; HDACa, HDAC-associated subunits; HMTc, histone methyltransferase (HMT) catalytic subunits; HMTa, HMT-associated subunits; and HDMTc, histone demethylase catalytic subunits.

FIGURE 3: Boxplots of combined evolutionary conservation levels of *Saccharomyces* and *Schizosaccharomyces* subunits of histone-modifying protein complexes. 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 $\leq 10^0$; score 3, $10^{-100} < E$ value $\leq 10^{-50}$; score 4, $10^{-150} < E$ value $\leq 10^{-100}$; score 5, E value $\leq 10^{-150}$. Each circle indicates the total of the scores of each protein. Boxes are composed of medians with first and third quartiles from the scores of the subunits. HATc, histone acetyltransferase (HAT) catalytic subunits; HATa, HAT-associated subunits; HDACc, histone deacetylase (HDAC) catalytic subunits; HDACa, HDAC-associated subunits; HMTc, histone methyltransferase (HMT) catalytic subunits; HMTa, HMT-associated subunits; HDMTc, histone demethylase catalytic subunits.

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 ($>.05$), 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

(Tables 1 and 2). 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.

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