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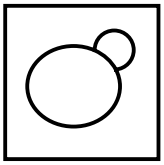

Research Article

In silico analysis for transcription factors with $Zn(II)_2C_6$ binuclear cluster DNA-binding domains in *Candida albicans*

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Abstract

A total of 6047 open reading frames in the *Candida albicans* genome were screened for $Zn(II)_2C_6$-type zinc cluster proteins (or binuclear cluster proteins) involved in DNA recognition. These fungal proteins are transcription regulators of genes involved in a wide range of cellular processes, including metabolism of different compounds such as sugars or amino acids, as well as multi-drug resistance, control of meiosis, cell wall architecture, etc. The selection criteria used in the sequence analysis were the presence of the $CysX_2CysX_6CysX_{5-16}CysX_2CysX_{6-8}Cys$ motif and a putative nuclear localization signal. Using this approach, 70 putative $Zn(II)_2C_6$ transcription factors have been found in the genome of *C. albicans*.

Keywords: ***Candida albicans*; transcription factor; $Zn(II)_2C_6$ domain; binuclear cluster proteins**

Introduction

Biological systems contain an important group of proteins characterized by their ability for DNA binding and participation in important processes, such as DNA replication and repair and transcription gene control. Gene expression can be controlled at various levels, including transcription, mRNA splicing, mRNA stability, translation and even post-translation events, such as protein stability and modification. There are many regulatory sequences in genes that bind various transcription factors. These regulatory sequences are essentially located upstream (5′) of the transcription initiation site, although some elements occur downstream (3′) or even within the genes themselves. The number and type of regulatory elements are variable for each gene. Moreover, various cell types express characteristic combinations of transcription factors; this is the major mechanism for cell-type specificity in the regulation of gene expression.

Transcription factors have been grouped in different families as a function of their DNA binding domains. These DNA binding domains comprise the fungal helix–loop–helix (HLH), helix–turn–helix (HTH), high mobility group (HMG) box, basic region–leucine zipper (bZIP), MADS box, TEA/ATTS domain, the zinc(II) coordinating Cys_2His_2, $Cys_2X_{17}Cys_2$ (GATA), copper DNA binding, heat shock transcription factor (HSTF) and $Zn(II)_2Cys_6$ binuclear cluster (Klug and Rhodes, 1987; Davis and Hynes 1987; Furst *et al*., 1988; Nehlin and Ronne, 1990; Burglin, 1991; Jakobsen and Pelham, 1991; Todd and Andrianopoulos, 1997; Kohler *et al*., 2002).

In fungi such as *Saccharomyces cerevisiae* (Pan and Coleman 1990; Akache *et al*., 2001), *Aspergillus flavus* (Woloshuk *et al*., 1994), *A. nidulans* (Ascone *et al*., 1997), *Fusarium solani* (Li and Kolattukudy, 1997), *A. niger* (Todd *et al*., 1997), *Kluyveromyces lactis* (Breunig and Kuger, 1987), *Neurospora crassa* (Yuan *et al*., 1991) and

Schizosaccharomyces pombe (Tang *et al.*, 1994), an important set of transcription factors is composed by a sub-family of zinc finger proteins named zinc cluster proteins or binuclear cluster proteins, $Zn(II)_2C_6$, characterized by containing the well-conserved motif $CysX_2CysX_6CysX_{5-16}CysX_2CysX_{6-8}Cys$ (Figure 1), with cystein residues binding to two zinc atoms which coordinate folding of the domain (Vallee *et al.*, 1991). In *C. albicans* only four $Zn(II)_2C_6$ proteins have been reported (Kelly and Kwon-Chung, 1992; Whiteway *et al.*, 1992; Talibi and Raymond, 1999, Moreno *et al.*, 2003). In the present study, we have taken a sequence-dependent approach to identify new $Zn(II)_2C_6$ ORFs by screening the genome database of *C. albicans* for $Zn(II)_2C_6$ transcription factors by an *in silico* analysis.

Materials and methods

The BLAST utility provided by the *C. albicans* genome database (**http://www.pasteur.fr/recherche/unites/GalarFungail**; Altschul *et al.*, 1997) was used to search for putative transcription factors containing the $Zn(II)_2C_6$ binuclear motif. The *C. albicans* putative $Zn(II)_2C_6$ proteins were aligned using the ClustalW online interface (**http://www.ebi.ac.uk/clustalw**; Thompson *et al.*, 1994) and manual alignment. After the alignment, the output data were submitted to the Phylip drawtree web interface utility at the Institute Pasteur (**http://bioweb.pasteur.fr/seqanal/interfaces/drawgram.html**; Lim and Zhang, 1999) to get the phenogram. Comparative analysis between *S. cerevisiae* and *C. albicans* $Zn(II)_2C_6$ putative transcription factors was carried out by reciprocal analysis of the SGD (**http://www.yeastgenome.org**) and *C. albicans* database entries. SCANPROSITE (**http://www.expasy.org/tools/scanprosite**; Gattiker *et al.*, 2002) was also used for proteins matching the consensus sequence. PSORTII (**http://psort.ims.u-tokyo.ac.jp**; Horton and Nakai, 1997) was used for subcellular localization prediction. The potential of dimerization via $Zn(II)_2C_6$ structures was investigated using the COILS program (**http://www.ch.embnet.org/software/COILS_form.html**; Lupas *et al.*, 1991) as described by Taylor and Zhulin (1999).

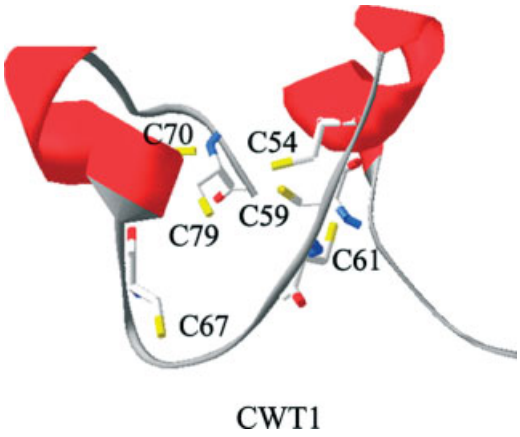

Figure 1. Schematic representation of a characteristic *C. albicans* $Zn(II)_2C_6$ domain. The structure was produced by threading it to the *S. cerevisiae* Cyp1 (Hap1) DNA binding domain (PDB: 1PYC) by using the JIGSAW utility (**http://www.bmm.icnet.uk/servers/3djigsaw**; Bates and Sternberg, 1999). The position of the six cysteines is annotated. The figure was generated using the Swiss Pdb-viewer (Guex and Peitsch, 1997)

Results and discussion

In silico screening for potential $Zn(II)_2C_6$ transcription factors

The determination of the complete genomic sequence of *Candida albicans* (**http://www-sequence.standford.edu/group/candida**), annotated by the European Consortium Galar Fungail (**http://www.pasteur.fr/recherche/unites/GalarFungail**), has allowed us to search for new putative transcription factors containing the $Zn(II)_2C_6$ binuclear motif. The criterion used for selection was the presence of the $CysX_2CysX_6CysX_{5-16}CysX_2CysX_{6-8}Cys$ cysteine pattern. All the 6047 *C. albicans* ORFs were screened, based on this criterion, and a set of 70 potential $Zn(II)_2C_6$ transcription factors, including the four previously known $Zn(II)_2C_6$ proteins, viz. CaFcr1p, CaSuc1p, CaCzf1p and CaCwt1p, was generated (Table 1). In the complete genome of *S. cerevisiae* a total of 58 $Zn(II)_2C_6$ proteins have been reported (Akache *et al.*, 2001).

Structure of the $Zn(II)_2C_6$ domain

The characteristic DNA binding domain of $Zn(II)_2C_6$ proteins contains a highly-conserved $CysX_2CysX_6CysX_{5-16}CysX_2CysX_{6-8}Cys$ motif, which was first described in *S. cerevisiae* (Pan and Coleman, 1990). In this motif the six cysteine residues

Table 1. Alternative names for $Zn(II)_2Cys_6$ proteins used in Candida DB (*C. albicans* DataBase at **http://genolist.pasteur.fr/CandidaDB**), SGTC (Stanford Genome Technology Center at **http://www-sequence.sandford.edu**) and CYGD (Comprehensive Yeast Genome Database at **http://pedant.gsf.de**)

Candida DB	SGTC	CYGD
IPF100.3	orf19.5940	CA6113
IPF376	orf19.7518	CA5860
IPF776	orf19.5338	CA5497
IPF907	orf19.7583	CA5985
IPF928	orf19.7570	CA5976
IPF1034	orf19.4573	CA1083
IPF1040	orf19.4568	CA1544
IPF1196	orf19.2077	CA4820
IPF1264	orf19.7371	CA5669
IPF1266	orf19.7372	CA5670
IPF1292	orf19.7381	CA5678
IPF1457	orf19.6038	CA4901
IPF1960.5f	orf19.7318	CA5554
IPF2029	orf19.5251	CA4996
IPF2319	orf19.6680	CA4282
IPF3444	orf19.6182	CA3201
IPF3781(CWT1)	orf19.5849	CA2880
IPF4835	orf19.5992	CA6071
IPF6159	orf19.1035	CA1038
IPF6203	orf19.4166	CA3716
IPF6510	orf19.1685	CA2306
IPF6554	orf19.4450	CA4663
IPF6874.3f	orf19.4251	CA2184
IPF7221	orf19.4046	CA2491
IPF7289	orf19.391	CA3878
IPF7629	orf19.1168	CA1786
IPF7952	orf19.3305	CA4614
IPF8224	orf19.5380	CA2298
IPF9188	orf19.3187	CA2539
IPF9251	orf19.5133	CA3639
IPF9312	orf19.4649	CA1718
IPF9499	orf19.2808	CA2621
IPF9826	orf19.4145	CA3088
IPF10079	orf19.2280	CA0257
IPF10197	orf19.2753	CA1892
IPF10533	orf19.1255	CA3454
IPF11777	orf19.4778	CA0777
IPF13021	orf19.2647	CA1726
IPF13024	orf19.2646	CA2064
IPF13158	orf19.5729	CA2844
IPF13229	orf19.3876	CA3551
IPF13264	orf19.2748	CA1171
IPF14113	orf19.166	CA0465
IPF14255	orf19.4767	CA1174
IPF15273	orf19.1822	CA0423
IPF15350	orf19.2745	CA0215
IPF16067	orf19.3190	CA2542
IPF16368.5f	orf19.255	CA0153
IPF19614	orf19.1496	CA1859
IPF19769	orf19.1718	CA2799
IPF19850	orf19.1227	CA0208
IPF19920	orf19.4524	CA1509
IPF20023	orf19.6985	CA5031
IPF20024	orf19.3012	CA5048
ARG81	orf19.4766	CA1175
CAT8	orf19.5097	CA2219
CTA7	orf19.4288	CA3060
CZF1	orf19.3127	CA3560
DAL81	orf19.3252	CA5449
ECM22	orf19.2623	CA0471
FCR1	orf19.6817	CA5890
LEU3	orf19.4225	CA4146
LYS14	orf19.5548	CA0404
PPR1	orf19.3986	CA4758
PUT3	orf19.6203	CA3214
RGT1	orf19.2747	CA1172
SEF1	orf19.3753	CA2346
SEF11.5eoc	orf19.1926	CA0395
STB5	orf19.3308	CA4617
SUC1	orf19.7319	CA5555

are responsible for maintaining the structure by binding two atoms of zinc (Todd and Andrianopoulos, 1997). Cys_1 and Cys_4 act by binding two zinc ions, whereas the remaining cysteine residues are terminal ligands (Figure 2) (Pan and Coleman, 1990).

The metal-binding domain is composed of two substructures with three cysteine residues in each one. Cys_1-Cys_2 and Cys_4-Cys_5 are canonically separated by two amino acid residues, while Cys_2-Cys_3 by six amino acid residues. Cys_3-Cys_4 separation is highly variable (5–16 amino acid residues) while Cys_5-Cys_6 separation has a length of 6–8 amino acid residues. The 70 OFRs found in *C. albicans* as putative $Zn(II)_2C_6$ proteins were aligned using the ClustalW program (Thompson *et al*., 1994); 25 of them exactly fit with the most restrictive pattern $CysX_2CysX_6CysX_6CysX_2CysX_6$ Cys.

Another important amino acid residue in DNA binding is a lysine residue localized between Cys_2 and Cys_3 (Figure 2). In some *S. cerevisiae* $Zn(II)_2C_6$ proteins such as Gal4p and Pdr1p (Laughon and Gesteland, 1984), this lysine residue is responsible for the specific contact with the CGG triplet in the DNA. This lysine residue is conserved in 57 of the *C. albicans* $Zn(II)_2Cys_6$ putative transcription factors (Figure 2), whereas 13 sequences contain arginine or histidine instead.

The subregion between Cys_3 and Cys_4 is highly variable. Although most of the motifs have a six

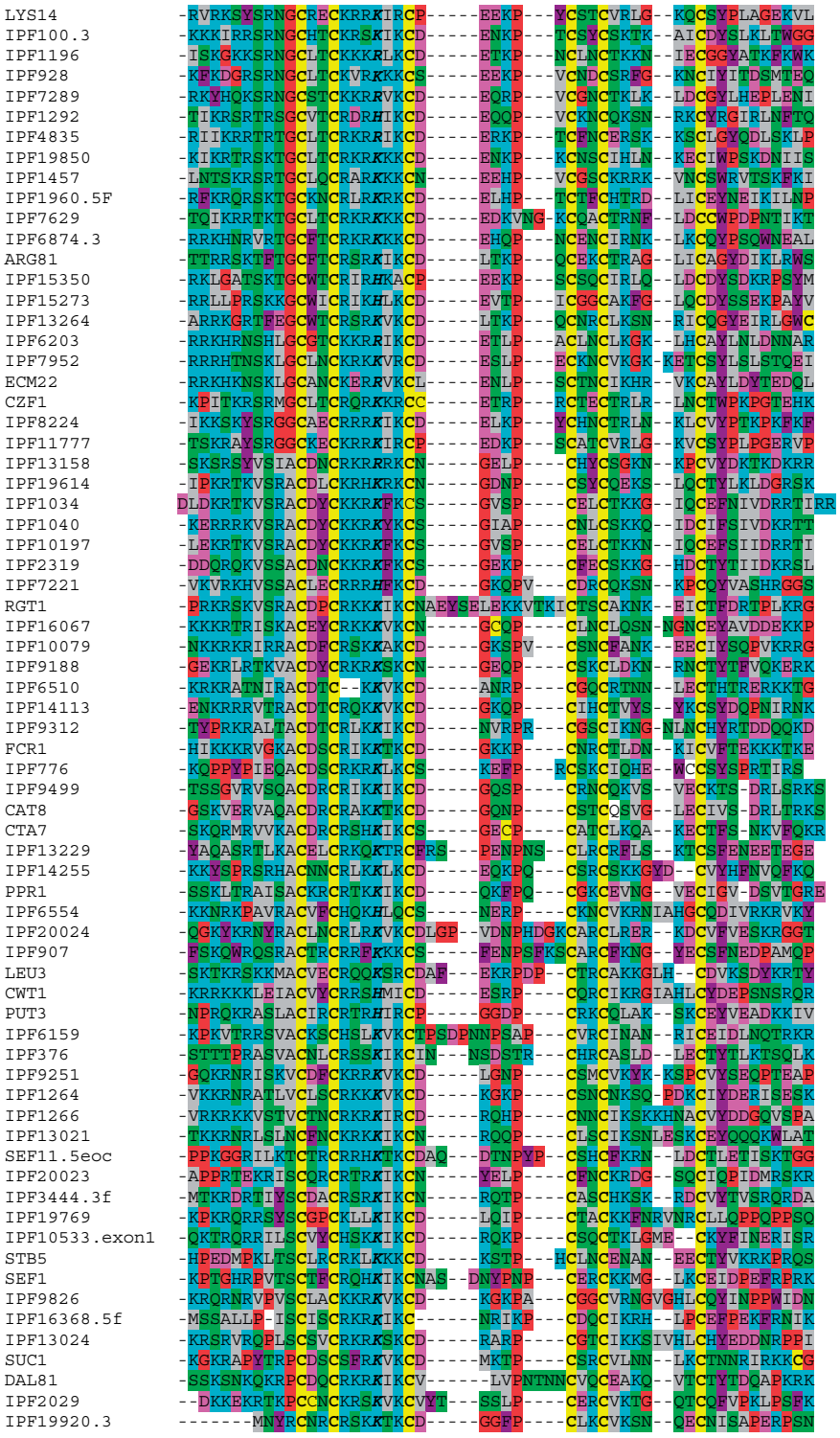

Figure 2. Alignment of the C_6 zinc cluster region in *C. albicans* proteins. The colour code is as follows: Cys, yellow and highlighted; Arg, His and Lys, blue (residues making specific DNA contact are also highlighted and in italics); Met, Val, Leu, Ala and Ile, grey; Glu, Asp, pink; Phe, Tyr and Trp, purple; Gln, Ser, Asn and Thr, green; Gly and Pro, red

amino acid residues extension, its variability ranges from five to 16 residues. A proline residue is present in almost all the ORFs identified (Figure 2), maybe involved in the turn required between the two α-helix subregions. The subregions between Cys_2 and Cys_3 and between Cys_5 and Cys_6 are rich in basic amino acids. A consensus sequence in the N- and C-terminal regions flanking the six cysteines domain has not been found. However, there is a predominance of basic amino acid residues (Lys and Arg) at both the N-terminus and at the C-terminus, but to a lesser extent. These basic domains could permit or enhance the DNA recognition.

Structure of the *C. albicans* $Zn(II)_2C_6$ proteins

$Zn(II)_2C_6$ transcription regulator factors are composed of two clearly different domains responsible for DNA binding and activation (Todd and Andrianopoulos, 1997, and references herein). In *C. albicans*, the $Zn(II)_2C_6$ domain is usually found at the N-terminal region of the protein with a few exceptions: five at the C-terminus and one in the middle of the protein (Figure 3).

Whiteway *et al*. (1992) identified the gene *CFZ1* that conferred moderate pheromone resistance on *S. cerevisiae*. Czf1p shows an overall structure that resembles that of a transcription factor, with a glutamine-rich region in the central part and a cysteine-rich region at the C-terminus of the protein.

The similarity outside the zinc cluster region reported for some *S. cerevisiae* $Zn(II)_2C_6$ proteins has also been found in *C. albicans*. It has been discovered that some putative fungal proteins contain a characteristic domain involved in transcription control (**http://www.sanger.ac.uk/cgi-bin/Pfam/getacc?PF04082**), although their function has not been elucidated to date (Marczak and Brandriss, 1991; Hedges *et al*., 1995; Kasten and Stillman, 1997; van Peij *et al*., 1998). The search in the *C. albicans* database for $Zn(II)_2C_6$ proteins containing such fungal domain revealed the occurrence of at least 12 ORFs (Figure 3). Moreover, some of these ORFs (PUT3, STB5, CAT8, PPR1, IPF20023 and DAL81) present a high level of identity with their respective *S. cerevisiae* homologues.

SCANPROSITE analysis (Gattiker *et al*., 2002) revealed the presence of other interesting motifs (Figure 3): (a) glutamine-rich regions, which may form hydrogen bonds with target factors (Courey and Tjian, 1988), resembling those previously described for other transcription factors (Aro *et al*., 2001); (b) proline-rich regions which may fold into a unique structure that forms protein–protein contact with the transcription machinery (Mermod *et al*., 1989) — IPF10079 and IPF9499 present such region at the C-terminus and RGT1 and IPF13024 at the N-terminus of the protein, close to the $Zn(II)_2C_6$ motif; (c) histidine, serine and threonine-rich regions (Figure 2); (d) basic leucine zipper domains, frequent in both *S. cerevisiae* (Fernandes *et al*., 1997) and *C. albicans* (Yang *et al*., 2001). These motifs are implicated in protein dimerization (Busch and Sassone-Corsi, 1990).

The global analysis of these proteins using the PSORTII program (Horton and Nakai, 1997) exhibits the presence of a nuclear localization signal (Talibi and Raymond, 1999; Moreno *et al*., 2003) in most of them, suggesting a putative nuclear localization (Table 2).

The presence of coiled-coil elements was searched for the 70 sequences by the COIL program (Taylor and Zhulin, 1999), to investigate the potential of dimerization via this structure. The program described by Lupas *et al*. (1991) assigns a score to each amino acid residue included in a window with 7, 14 or 28 residues (two, three or four heptads) on the basis of their probability of being involved in a coiled-coil structure. Positive scores were only reported when probability values were >0.9 in the 150 residues of the C-terminal $Zn(II)_2C_6$ domain (Table 2). From the 65 putative proteins with an N-terminal $Zn(II)_2C_6$ domain, a high peak was detected in 28, 21 and 17 for a two-, three- or four-heptad window, respectively. This analysis shows that the occurrence of a coiled-coil region situated at the C-terminus of the $Zn(II)_2C_6$ domain is quite frequent in these putative transcription factors, and is probably involved in dimerization events.

A transcription factor (CaCwt1p), required for cell wall integrity, has been recently characterized by our group (Moreno *et al*., 2003). CaCwt1p has been structurally analysed and the presence of another family of C-terminal motifs has been predicted. This region, named PAS, is presumed to be involved in eukaryotic signal transduction or dimerization events (Taylor and Zhulin, 1999). The

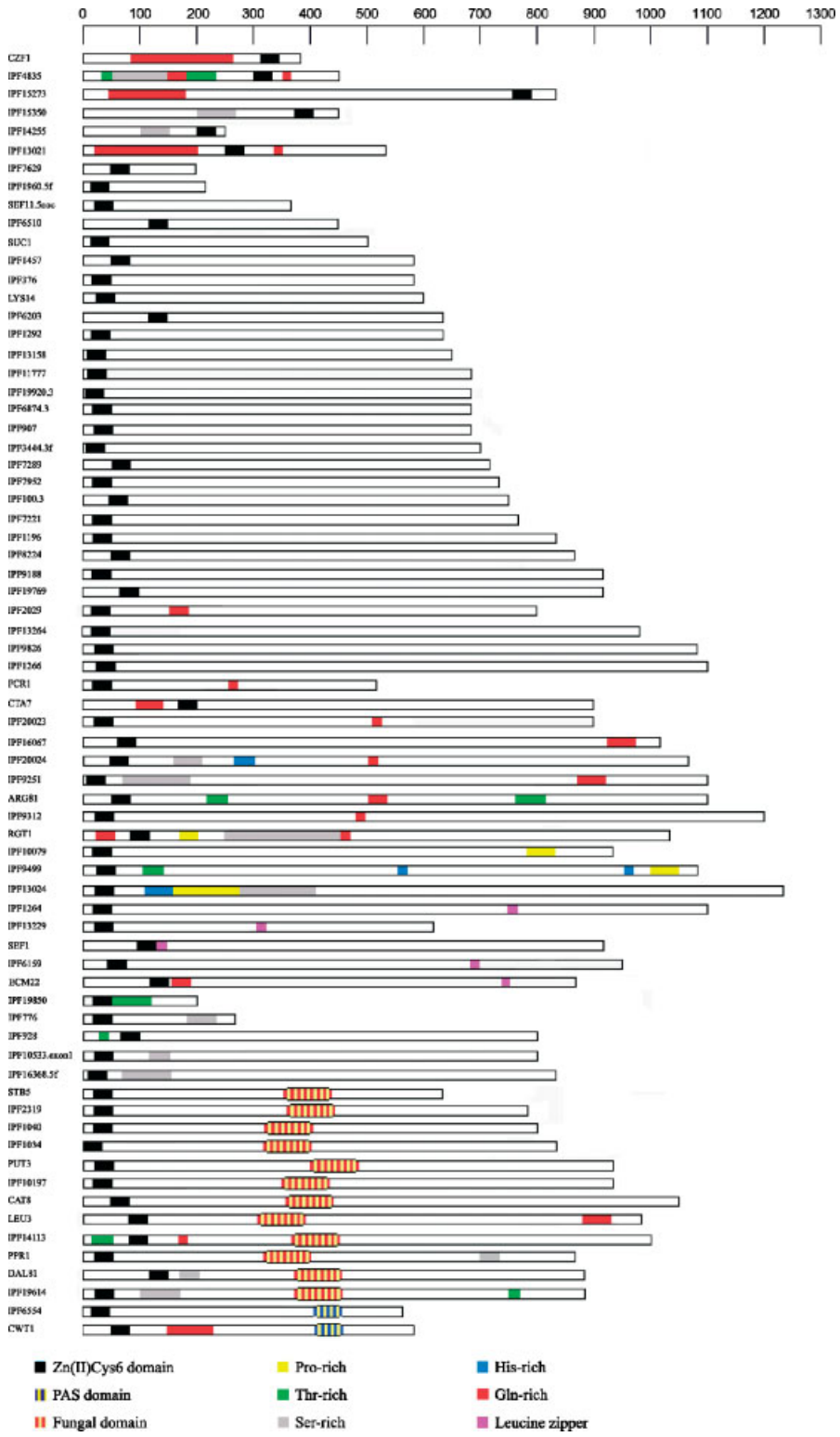

Figure 3. Schematic structural representation of the most common structural features predicted for the *C. albicans* $Zn(II)_2Cys_6$ protein family

Table 2. Summary of data for the *Candida albicans* $Zn(II)_2Cys_6$ cluster proteins

ORF name	Nuclear localization (%)	Last Cys	Coiled-coil probability >0.9 (within 150 residues)		
			Two heptads	Three heptads	Four heptads
IPF16368.5f	94.1	36	+	+	+
IPF19850	94.1	45	–	–	–
IPF15350	94.1	Ct	na	na	na
IPF10079	94.1	45	–	–	–
SEF11.5eoc	56.5	59	–	–	+
LYS14	4.3	67	–	–	–
IPF15273	87.0	Ct	na	na	na
IPF14113	69.6	61	–	–	–
ECM22	82.6	143	+	+	+
IPF11777	95.7	40	–	–	–
IPF6159	73.9	86	+	+	+
IPF1034	94.1	42	–	–	–
IPF13264	31.8	44	+	+	–
RGT1	69.6	125	–	–	–
IPF14255	78.3	Ct	na	na	na
ARG81	52.2	135	+	–	–
IPF19920.3	13.0	31	–	–	–
IPF1040	100.0	43	–	–	–
IPF9312	21.7	267	–	–	–
IPF13021	65.2	121	–	–	–
IPF7629	87.0	84	–	–	–
IPF19614	43.5	57	–	–	–
IPF10197	21.7	41	+	–	–
IPF13024	82.6	61	–	–	–
IPF6874.3	21.7	51	–	–	–
CAT8	65.2	80	+	+	+
IPF8224	65.2	71	–	–	–
IPF6510	65.2	141	–	–	–
SEF1	73.9	120	+	+	+
IPF9188	52.2	52	+	+	+
IPF16067	43.5	93	+	+	+
IPF9499	69.6	58	+	+	+
IPF19769	21.7	97	+	+	+
IPF13158	26.1	39	–	–	–
CWT1	78.3	79	–	–	–
CTA7	73.9	201	–	–	–
IPF9826	43.5	67	+	+	+
IPF3444.3f	0.0	37	+	–	–
PUT3	69.6	57	+	+	+
IPF10533.exon1	65.2	74	+	+	+
IPF13229	65.2	74	–	–	–
CZF1	73.9	Ct	na	na	na
IPF9251	39.1	39	+	+	–
IPF6203	82.6	155	–	–	–
IPF7289	65.2	141	–	–	–
IPF7221	73.9	48	–	–	–
LEU3	65.2	112	+	+	–
IPF2319	17.4	57	+	+	+
IPF7952	43.5	46	–	–	–
STB5	22.2	66	–	–	–
IPF6554	95.7	48	–	–	–
PPR1	69.6	62	+	–	–
IPF1196	69.6	55	–	–	–

Table 2. Continued

ORF name	Nuclear localization (%)	Last Cys	Coiled-coil probability >0.9 (within 150 residues)		
			Two heptads	Three heptads	Four heptads
IPF1457	87.0	78	–	–	–
IPF2029	69.6	45	+	+	–
IPF20023	43.5	68	+	–	+
IPF20024	60.9	78	–	–	–
DAL81	65.2	149	–	–	–
IPF776	69.6	50	+	–	–
IPF1960.5f	65.2	44	–	–	–
SUC1	47.8	39	–	–	–
IPF1264	69.6	50	+	+	+
IPF1266	47.8	59	+	+	+
IPF1292	65.2	46	–	–	–
IPF376	11.1	43	–	–	–
FCR1	82.6	52	+	+	–
IPF928	21.7	101	+	+	–
IPF907	39.1	55	+	–	–
IPF4835	56.5	Ct	na	na	na
IPF100.3	21.7	70	–	–	–

Ct, located at the C-terminus of the protein.
Nuclear localization was predicted with PSORT-II (Horton and Nakai, 1997). Coiled-coil probability within the C-terminal region of the $Zn(II)_2Cys_6$ domain was calculated by COILS (Lupas *et al.*, 1991).

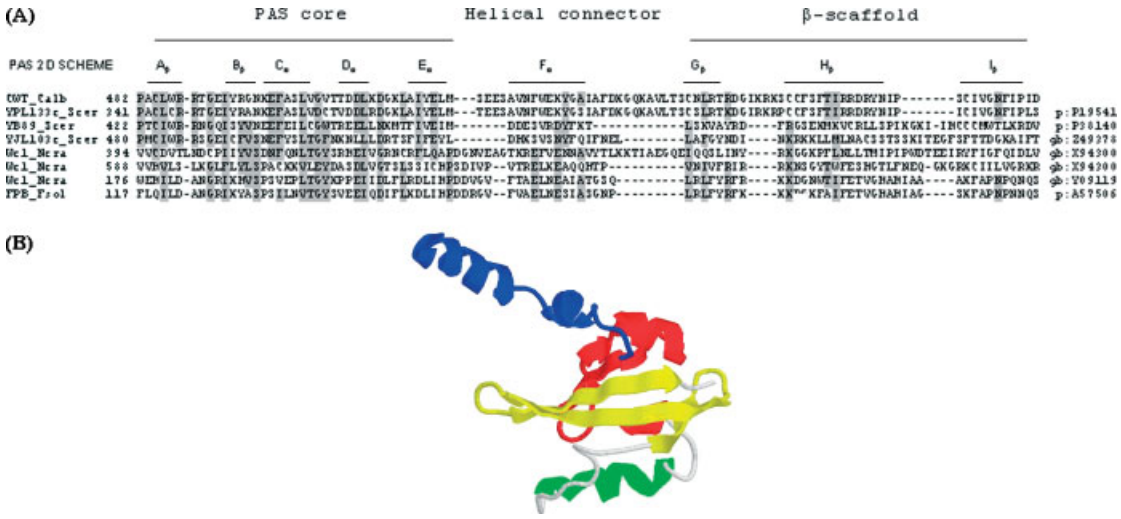

Figure 4. (A) Multiple structure-based alignment of fungal PAS domains. The alignment was performed as described by Taylor and Zhulin (1999), with modifications. (B) The secondary structure of CaCwt1p was deduced from that of *Rhizobium melitoti* FixL (PDB: 1ew0). Similar residues in at least four sequences are highlighted. The N-terminal link region (blue), PAS core (red), helical connector (green) and β-scaffold (yellow) are shown. The figure was generated using RasMol (Sayle and Milner-White 1995)

putative protein IPF6554 also possesses such characteristic domain. A multiple structure-based alignment also including the *S. cerevisiae* sequences for CaCwt1p, YPL133c and YJL103c, respectively, is shown in Figure 4. The biological function of the PAS domain in yeast proteins has not been elucidated yet. Although many of the transcription factors described at present can be basically depicted as three-component proteins, where the DNA recognition motif is linked to a dimerization region by a function variable spacer, this is not a general rule. This general structure is not always

Table 3. Comparison between genes encoding putative zinc cluster proteins in *S. cerevisiae* and *C. albicans*. Only positive results from reciprocal BLAST are included

Saccharomyces cerevisiae				*Candida albicans*	
Systematic name	**Gene**	**Function**	**Ref.**	**Systematic name**	**Ref.**
YDR207c	UME6	Regulator of early meiotic genes	a	IPF15350	—
YDR034c	LYS14	Transcriptional activator of lysine pathway genes	b	LYS14	—
YIL130w	—	Unknown	—	IPF14113	—
YML076c	WAR1	Regulate weak acid stress response	c	IPF6159	—
YKL038w	RGT1	Constitutive expression of glucose-induced HXT genes	d	RGT1	—
YML099c	ARG81	Regulator of arginine responsive genes	e	ARG81	—
YBL066c	SEF1	Suppressor of essential function	e	SEF1	—
YPL133c	RDS2	Unknown	e	CWT1	i
YMR019c	STB4	Unknown	f	CTA7	—
YLR256c	HAP1	Regulator of oxygen-dependent genes	e	IPF9826	—
YKL015w	PUT3	Regulator of Pro utilization genes	g	PUT3	—
YDR520c	—	Unknown	—	IPF13229	—
YOR363c	PIP2	Activator of peroxisome proliferation	e	IPF9251	—
YDR213w	UPC2	Involved in sterol uptake	e	IPF7289	—
YLR451w	LEU3	Regulator of amino acid biosynthesis	e	LEU3	—
YHR178w	STB5	Binds Sin3p in two-hybrid assay	f	STB5	—
YJL103c	—	Unknown	h	IPF6554	—
YLR014c	PPR1	Activator of URA1 and URA3 genes	h	PPR1	—
YOR337w	TEA1	Activator of Ty1 elements	e	IPF20023	—
YDR421w	ARO80	Unknown	e	IPF20024	—
YIR023w	DAL8	Regulator of nitrogen catabolic genes	e	DAL81	—
YFL052w	—	Unknown	e	SUC1	—
YDL170w	UGA3	Unknown	h	IPF928	—

References: a, Tong *et al.* 2004; b, El Alami *et al.* 2002; c, Kren *et al.* 2003; d, Kim *et al.* 2003; e, Akache *et al.* 2001; f, Kasten & Stillman 1997; g, Axelrod *et al.* 1991; h, Giaever *et al.* 2002: i, Moreno *et al.* 2003.

present and some well-characterized proteins lack this dimerization motif (Anderson *et al.*, 1995).

The function of most of the *C. albicans* Zn(II)$_2$C$_6$ proteins remains uncharacterized, although a few cluster proteins, involved in several biological functions, have been reported. CaSuc1p is a transcription factor involved in sucrose utilization by affecting an inducible α-glucosidase, and was the first Zn(II)$_2$C$_6$ zinc finger protein described in *C. albicans* (Kelly and Kwon-Chung, 1992). The *CaCFR1* gene encoding a Zn(II)$_2$C$_6$ protein was isolated by its ability to complement the fluconazole hypersensitivity of a *S. cerevisiae* mutant lacking the transcription factors Pdr1p and Pdr3p (Talibi and Raymond, 1999). An atypical protein with a C-terminal Zn(II)$_2$C$_6$ motif, CaAzf1p, has also been reported previously (Whiteway *et al.*, 1992).

Some other *C. albicans* putative Zn(II)$_2$C$_6$ transcription regulators have also been tentatively assigned by comparison with their corresponding *S. cerevisiae* homologues (Table 3). However, the functional role of these similarities remains unclear, and could be related to evolutionary aspects. As an example, some zinc cluster proteins control the expression of genes required for gluconeogenesis in *S. cerevisiae*, such as Cat8p (Hedges *et al.*, 1995), Arg81p (Messenguy, 1976), Lys14p (Ramos *et al.*, 1988) and Ppr1p (Marmorstein and Harrison, 1994), involved in the metabolism of arginine, lysine and pyrimidines, respectively, which have homologues in *C. albicans* with a high sequence identity. Another member of the Zn(II)$_2$C$_6$ protein family, CaPut3p, involved in controlling enzymes required for proline use as a nitrogen source, was previously characterized in *S. cerevisiae* (Marczak and Brandriss, 1989).

Evolutionary relationships among Zn(II)$_2$C$_6$ clusters

Phylogenetic analysis was performed using the entire DNA binding region, including the Zn(II)$_2$C$_6$

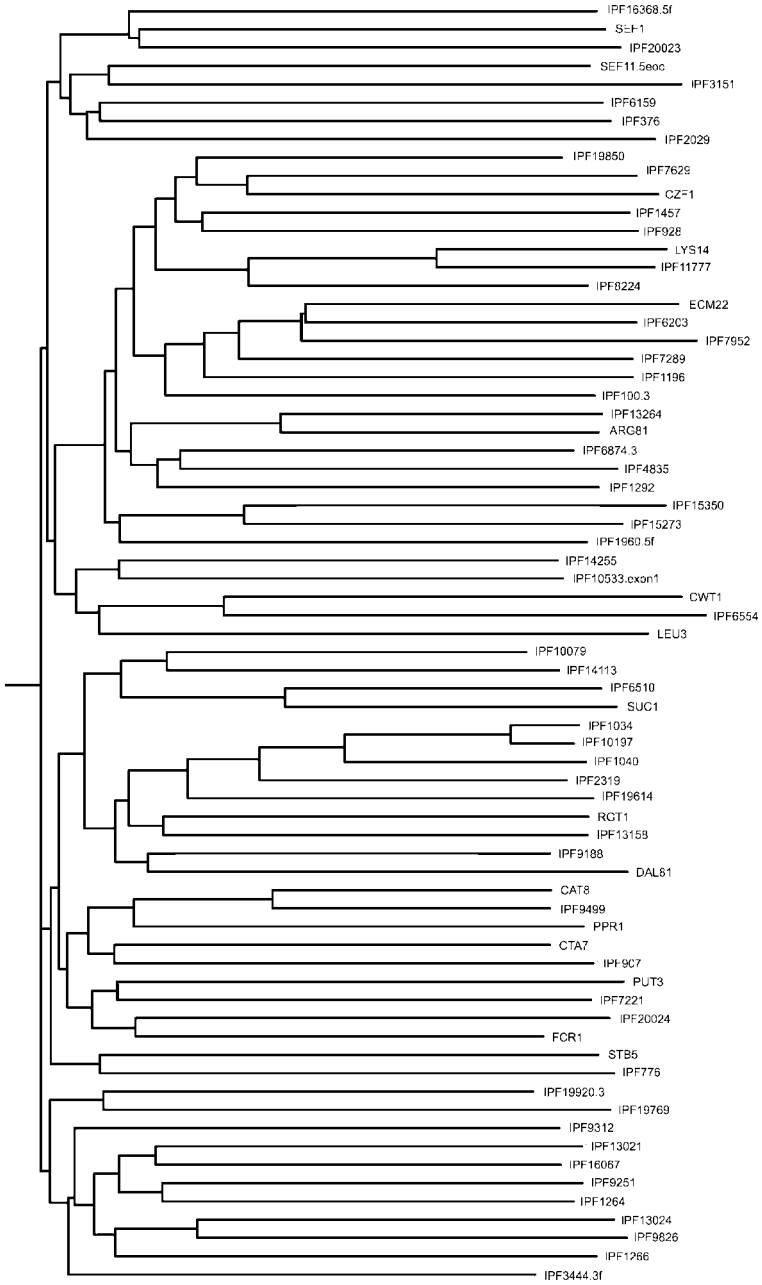

Figure 5. Phenogram of Zn(II)2Cys6 domains. Proteins containing $Zn(II)_2Cys_6$ domains have been obtained from the *C. albicans* genome database at **http://www-sequence.stanford.edu/group/candida**. The 10 N- and C-terminal flanking nucleotides were included within the input sequences

cluster and N- and C-terminal flanking sequences of the 70 predicted *C. albicans* proteins. After alignment using the ClustalW program (Figure 2), the output data were submitted to the Phylip drawtree web interface utility and a phenogram was obtained (Figure 5).

Sometimes there is strong support for grouping as inferred from bootstrap analysis. All the $Zn(II)_2C_6$ proteins containing the fungal domain previously described, with the single exception of LEU3, have been clustered in a single branch. This consistent association of unknown proteins

could represent regulation via a common pathway, although this remains to be elucidated. CWT1 and IPF6554 have also been consistently clustered on the basis of their $Zn(II)_2C_6$ domains. This data, together with the presence of the PAS domain in both these two putative proteins suggests their possible involvement in similar functional processes that should be investigated.

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