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A Fungal Family of Transcriptional Regulators: the Zinc Cluster Proteins

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INTRODUCTION

The trace element zinc is required for proper function of a large number of proteins, including various enzymes. However, most zinc-containing proteins are transcription factors capable of binding DNA and are named zinc finger proteins. They are categorized into various families according to zinc-binding motifs. For example, the Cys_2His_2 family comprises hundreds of zinc finger proteins that are found in eukaryotes ranging from yeast to humans. In contrast, members of the zinc cluster protein family (or binuclear cluster) are exclusively fungal and possess the well-conserved motif $CysX_2CysX_6CysX_{5-12}CysX_2CysX_{6-8}Cys$. The cysteine residues bind to two zinc atoms, which coordinate folding of the domain involved in DNA binding.

The family of zinc cluster proteins is best characterized for the budding yeast, *Saccharomyces cerevisiae*. The genome of this organism encodes over 50 known (or putative) zinc cluster proteins. The first- and best-studied zinc cluster protein is Gal4p, a transcriptional activator of genes involved in the catabolism of galactose. Zinc cluster proteins are also found in a variety of other fungal organisms, such as *Kluyveromyces lactis*, the fission yeast *Schizosaccharomyces pombe*, and the human pathogens *Candida albicans* and *Aspergillus nidulans*. This review is aimed at describing the structural and functional domains of zinc cluster proteins and summarizing their roles in fungal physiology as well as their modes of action in *S. cerevisiae* and other fungi.

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ZINC FINGER PROTEINS: AN OVERVIEW

Zinc-binding proteins form one of the largest families of transcriptional regulators in eukaryotes, displaying variable secondary structures and enormous functional diversity. They are grouped together because they all harbor at least one common motif, the zinc finger. This motif was first identified in the *Xenopus* transcription factor TFIIIA 20 years ago (179), and the resolution of its three-dimensional solution structure a few years later revealed its protruding "finger-like" shape (145). The finger actually consists of one α helix and a pair of antiparallel β strands (287). In general, one or more zinc atoms are bound by cysteine or histidine residues. This stabilizes the domain and contributes to proper protein structure and function (135, 287). The majority of zinc finger proteins bind to DNA (and also to RNA in the case of TFIIIA), thereby playing important roles in transcriptional and translational processes (135). However, it should be noted that this superfamily of proteins is not solely restricted to binding nucleic acids. Newly

TABLE 1. Three major classes of eukaryotic zinc finger proteins

Zinc finger class	Subclass(es)	Consensus amino acid sequence	Example
I (C_2H_2)	FOG (C_2HC)	Cys-X_{2-4}-Cys-X_{12}-His-X_{3-5}-His	*Xenopus* TFIIIA
II (C_4)	GATA, nuclear receptors, LIM (C_3H)	Cys-X_2-Cys-X_n-Cys-X_2-Cys-X_n-Cys-X_2-Cys-X_n-Cys-X_2-Cys	Glucocorticoid receptor
III (C_6)		Cys-X_2-Cys-X_6-Cys-X_{5-12}-Cys-X_2-Cys-X_{6-8}-Cys	*S. cerevisiae* Gal4p

identified zinc finger proteins are also involved in many other physiological roles, including mediating protein-protein interactions, chromatin remodeling, protein chaperoning, lipid binding, and zinc sensing (135). Of the DNA (or RNA)-binding variety, three major classes of zinc finger proteins have been established to date in eukaryotes, based on their unique and highly conserved consensus amino acid sequences. They are summarized in Table 1. Although they can be grouped together as zinc-binding transcription factors, each class has distinct structural properties.

Classes of Zinc Finger Proteins

Class I encompasses the Cys_2His_2 (C_2H_2) proteins and is often referred to as the classical zinc finger (reviewed in reference 287). It is one of the most common types of transcription factors found in eukaryotes, and these proteins contain two or more repeating zinc finger units. A well-known example in humans is the transcription factor Sp1 (152, 186). FOG proteins (*f*riend *o*f *G*ATA) are a subclass within this group because they contain standard zinc fingers (C_2H_2) along with a C_2HC consensus sequence (265). Each repeating unit consists of a conserved amino acid sequence that interacts with one zinc atom. Moreover, members of this class binds to nucleic acids as monomers (135, 155).

Class II represents the Cys_4 (C_4) zinc fingers, which include the GATA, LIM, and nuclear receptor proteins. GATA transcription factors (GATA-1 to -6) bind to a DNA sequence called a GATA motif [(A/T)GATA(A/G)] in the regulatory regions of their target genes through two zinc finger domains (270). The mammalian glucocorticoid receptor represents an excellent example within this class (39); its structure has provided much information on the DNA-binding capabilities of this group. Unlike the first class, these proteins usually contain one zinc finger unit binding to DNA as homodimers or heterodimers (consisting of two C_4 proteins). Usually, homodimers recognize inverted repeats within the target nucleic acid sequence, whereas heterodimers bind to direct repeats (135).

Class III (C_6) zinc finger proteins contain a DNA-binding domain (DBD) that consists of six cysteine residues bound to two zinc atoms, and hence these have the names zinc cluster, zinc binuclear cluster, or $Zn(II)_2Cys_6$ (Zn_2C_6) proteins. This class of transcription factors is unique in that these proteins contain only one zinc finger unit that binds two zinc atoms. They may interact with DNA as monomers, homodimers, or heterodimers (156, 233, 260, 267). Furthermore, they are strictly fungal proteins. The *Saccharomyces cerevisiae* transcription factor Gal4p is arguably the most well-known and well-studied zinc cluster protein. Its classification as a zinc "cluster" protein and the resolving of its X-ray crystal structure over a decade ago (168, 199) became the driving force behind studies which further characterized it and other members within this fungal superfamily of transcription factors.

ZINC CLUSTER PROTEINS

As stated above, the zinc binuclear cluster proteins (hereafter referred to as zinc cluster proteins) have been identified exclusively in fungi, although the other classes of zinc fingers are also present in this kingdom. For example, Msn2p, Msn4p, and Adr1p are all yeast transcription factors that contain a class I C_2H_2 motif (79). Zinc cluster proteins seem to belong predominantly to the ascomycete family, as only one (*Lentinus edodes*, PRIB protein) has been characterized in the basidiomycete family to date (61). Evolutionarily speaking, one hypothesis suggests that this unique $Zn(II)_2Cys_6$ motif appeared prior to the divergence of these two major fungal groups (260). Importantly, a multitude of recently identified zinc cluster proteins in *Aspergillus*, *Candida*, and *Saccharomyces* species, as well as *Schizosaccharomyces pombe*, are being studied (see Tables 3 to 5). The list of known zinc cluster proteins is growing rapidly, and the sequencing of other fungal genomes will allow for the identification of more transcription factors within this superfamily.

Structural and Functional Domains

Like most transcription factors, zinc cluster proteins contain several functional domains apart from the cysteine-rich DBD, including the regulatory and activation domains. A model depicting functional domains is shown in Fig. 1.

The entire DBD is separated into three regions: the zinc finger, linker, and dimerization regions. Pioneer work done on Gal4p (activator of *GAL* genes) and Ppr1p (activator of *URA* genes) has elucidated much of the structural biology of these transcription factors. The metal-binding portion of the DBD is described as having two substructures; each is formed by three cysteines that are surrounded on both sides with basic amino acids and are separated by a loop (233). Together, these form a pair of short alpha helices, between which are nestled two zinc atoms bound and bridged by a total of six cysteine residues

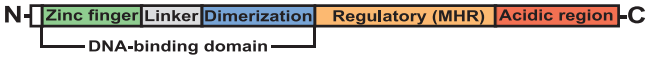

FIG. 1. Functional domains of zinc cluster proteins. Zinc cluster proteins can be separated into three functional domains: the DBD, the regulatory domain, and the acidic region. In addition, the DBD is compartmentalized into subregions: the zinc finger, the linker, and the dimerization domain. These regions contribute to DNA-binding specificity and to protein-DNA and protein-protein interactions (267). MHR, middle homology region.

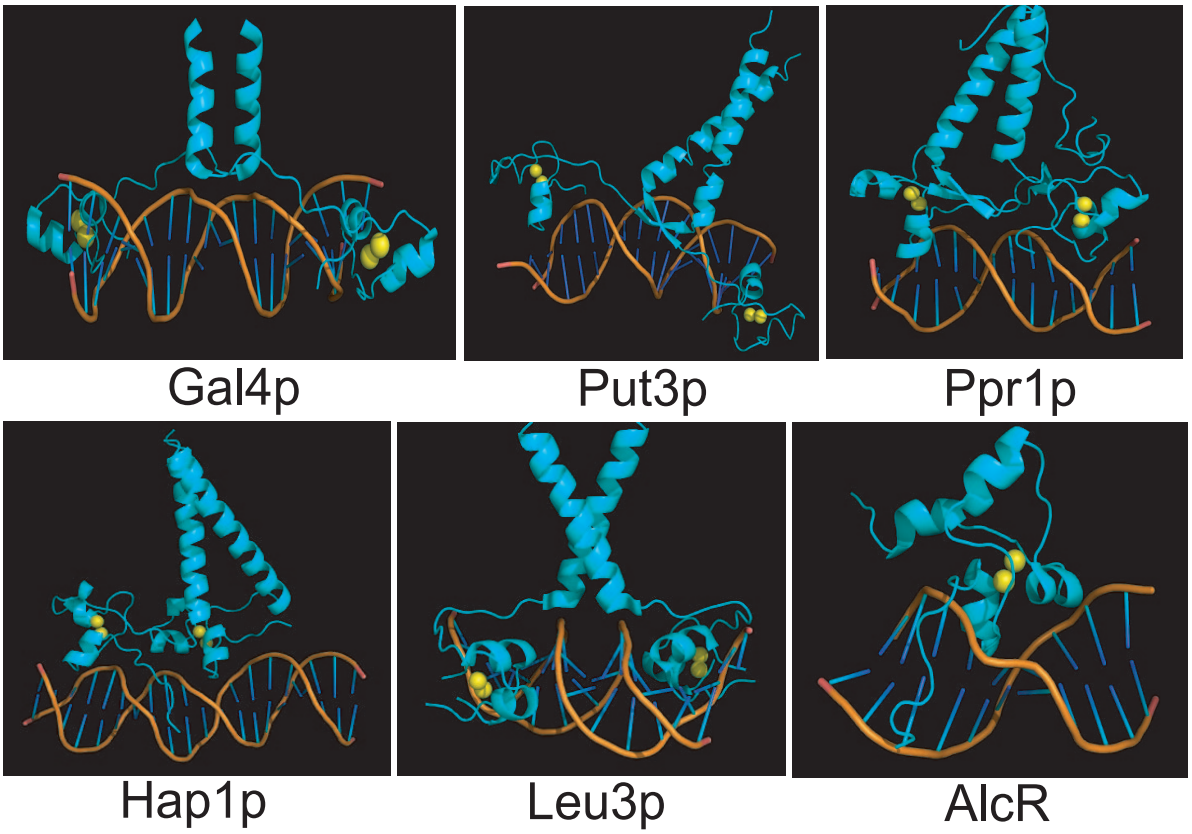

FIG. 2. Crystal structures of the DBDs of some $Zn(II)_2Cys_6$ regulators. AlcR binds as a monomer (28), while Gal4p, Put3p, Ppr1p, Leu3p, and Hap1p bind as homodimers. Gal4p, Put3p, and Ppr1p recognize inverted DNA repeats (168, 169, 253, 278). Leu3p and Hap1p bind to everted and direct repeats, respectively (72, 89, 98, 124, 294). Yellow spheres correspond to zinc atoms.

(78, 199). This cysteine-rich DBD is commonly located at the N terminus. However, at least two characterized C-terminal zinc cluster proteins also exist. They include *S. cerevisiae* Ume6p, as well as *C. albicans* Czf1p (283). Several mutagenic studies demonstrate the importance of the six cysteine residues in DNA binding and protein function (12, 50, 79, 111, 204, 205, 248, 260, 293). Other residues found within the metal-binding motif are equally important. For example, a conserved proline located in the loop between the two substructures provides flexibility (168), while a highly conserved lysine residue (sometimes replaced by arginine, histidine, or glutamine) is positioned between the second and third cysteines (168, 169, 233). X-ray crystallography of the *S. cerevisiae* Gal4p and Ppr1p DBDs performed by Marmorstein et al. (168) confirmed that these proteins bind as homodimers (Fig. 2). In fact, the cysteine-rich regions of these two proteins are remarkably similar. The zinc clusters of the homodimer complexes recognize a pair of CGG nucleotide triplets, interacting via major-groove contacts. This not only reflects the high degree of homology among members of this protein class but also suggests that other domains/factors must influence DNA targeting by these transcriptional regulators (see below).

At least two known zinc cluster proteins do not require the cysteine-rich DBD. Both *S. cerevisiae* Dal81p and *Aspergillus nidulans* TamAp proteins appear fully functional when their zinc clusters are deleted or disrupted (25, 49). Three other members of this superfamily in *S. cerevisiae* do not bind to DNA directly. The *RSC3* and *RSC30* genes encode proteins which make up part of the chromatin-remodeling complex RSC (*r*emodel the *s*tructure of *c*hromatin) (8), while Cep3p is an important component of the kinetochore complex (143, 250).

With a few exceptions, the requirement for zinc in stabilizing protein folding and function in this transcription factor class is obvious. However, several key experiments performed over a decade ago illustrate that zinc can be replaced by other metal ions, while still allowing for proper protein function. In determining the X-ray crystal structure of Gal4p, Marmorstein et al. showed that Cd^{2+}-containing crystals were of better quality than those containing Zn^{2+} ions (168). In addition, the nuclear magnetic resonance (NMR) solution structure of Ume6p was solved by demonstrating that zinc could also be replaced with cadmium (7). Importantly, both groups showed that these proteins bind to DNA in a metal ion-dependent manner.

The linker region is located C-terminally to the zinc cluster motif. It can take on very different forms, and sequence alignments show no similarities between linkers in various zinc cluster proteins. For example, the linker region for Gal4p extends along one DNA strand, contacting the phosphodiester backbone (168). In Ppr1p, the linker region is made up of antiparallel β sheets (169). Moreover, the Hap1p DBD also targets two CGG triplets, but in a direct-repeat orientation, as

opposed to the case for Gal4p, Put3p, and Ppr1p, where the CGG triplets are inverted (Fig. 2). The crystal structure of Hap1p shows that the protein dimer is asymmetrical and that the linker regions of both monomers interact exclusively with different residues, occupying two very different environments (124). Various linker regions between zinc cluster proteins that recognize similar nucleotides can explain this region's role in contributing to DNA-binding specificity. Replacing the zinc cluster motif of one protein with another does not affect DNA targeting, although switching linker regions does (166, 214). Moreover, mutations in the Gal4p and Ppr1p linker regions also affect DNA binding and proper protein function (111). It is, therefore, proposed that the linker region provides a rigid scaffold, mediating DNA binding to a preferred sequence and preventing binding to any alternate sites (166).

The dimerization region is the last element within the DBD and is typically positioned C terminal to the linker. The majority of zinc proteins contain this region, which is made up of heptad repeats similar to those found in leucine zippers (233). These heptad repeats form a highly conserved coiled-coiled structure which is most likely responsible for dimerization and protein-protein interactions. Importantly, this coiled-coil element is absent in *S. cerevisiae* Ume6p, which is one of the two characterized zinc cluster proteins containing a C-terminal DBD. This evidence suggests that Ume6p most likely acts as a monomer (202, 260).

The regulatory domain contains an important region displaying lesser homology among most members within this protein class, and it is termed the middle homology region. This region is what separates the DBD from the C-terminal acidic region. Although not always present in all zinc cluster proteins, this region, which spans about 80 amino acids, is thought to play a role in regulating the transcriptional activity of these factors (233, 267). This model is based on several studies in which the deletion of this region often renders zinc cluster proteins constitutively active. For example, removal of a region encompassing the middle homology region in *S. cerevisiae* Hap1p results in transcriptional activation even in the absence of the inducing molecule, heme; this suggests an additional role of oxygen sensing for this region in Hap1p as well (205). When a similar region is deleted in *S. cerevisiae* Leu3p, the protein is permanently activated (76, 297). Other examples include the Pdr1p and Pdr3p mutants that contain gain-of-function mutations within this region, implying that it possesses an inhibitory role (53, 128).

Most often C-terminally located, the acidic domain acts as an activation domain (233, 267). This is not a conserved domain, and its function/structure within this superfamily of transcriptional regulators is varied and not well defined. In both Pdr1p and Pdr3p, gain-of-function mutations have also been found in this motif (29, 196). Interestingly, several predicted transmembrane motifs are located in the activation domain of the *C. albicans* Upc2p (163, 243). This supports the hypothesis that this transcription factor may be membrane anchored in the cytoplasm prior to cleavage and translocation to the nucleus (243). Strangely, the deletion of only the last 10 C-terminal amino acids from Uga3p results in a totally inactive form of the transcription factor (M.-A. Sylvain and B. Turcotte, unpublished data). Clearly, this domain plays an important yet individualized role in each zinc cluster protein.

Binding Elements and DNA-Binding Specificity

Many studies show that zinc cluster proteins recognize highly related elements containing trinucleotide sequences in single or repeat forms, in either a symmetrical or an asymmetrical format. CGG triplets are common, although variations within these binding elements have also been reported (Table 2). Because these highly conserved transcriptional regulators all target very similar sequences, several strategies are needed to generate a vast repertoire of binding sites. This ensures that the required protein is able to carry out its own specific regulatory task (156, 233, 260, 267). As stated above, many factors influence DNA targeting and binding by zinc cluster proteins. In terms of protein structure, many components of the DBD contribute substantially in binding to target DNA. Moreover, nucleotides surrounding the CGG triplets also determine DNA-binding affinities to some extent (195). However, two very important determinants of DNA-binding specificity are the orientation of the CGG triplets and the spacing between these triplets.

Zinc cluster proteins can bind as homodimers to CGG triplets that are oriented in everted, inverted, or direct repeats. Gal4p, Put3p, and Ppr1p bind to inverted repeats whereby the zinc clusters of each monomer face each other (Fig. 2). Leu3p and Pdr3p exemplify how a homodimer binding to an everted repeat consists of two zinc clusters facing away from one another, while a Hap1p homodimer contains two zinc clusters facing the same direction in order to bind to a direct repeat (Fig. 2). Figure 3 represents a model illustrating this differential binding. Spacing between trinucleotide sequences is critical for zinc cluster proteins that bind to CGG triplets in the same conformation. For example, Gal4p binds to inverted CGG triplets spaced by 11 bp (CGG-N_{11}-CCG), whereas Put3p binds to CGGs separated by 10 bp (CGG-N_{10}-CCG) (11, 242, 272).

As more zinc cluster proteins are characterized, the presence of monomers and heterodimers predicts that realistically, many variations and combinations of this paradigm most likely occur. One possibility is that heterodimers comprised of members within this family bind preferentially to slightly different combinatorial elements, increasing the number of potential binding sites for this protein class. The physiological presence of Pdr1p and Pdr3p homodimers and Pdr1p/Pdr3p heterodimers in yeast provides evidence supporting this theory (167).

It remains to be seen whether or not other structures/motifs/binding elements may also be important in DNA recognition but have yet to be characterized. A recent large-scale study employing a powerful strategy for genome-wide location analysis demonstrates that many zinc cluster proteins can regulate other target genes through elements different from those initially identified (92). The technique combined the chromatin immunoprecipitation (ChIP) of approximately 200 tagged transcriptional regulators in *S. cerevisiae* (including many zinc cluster proteins) with DNA microarrays consisting of all the intergenic sequences within the yeast genome. This approach is useful when identifying additional targets for one transcriptional regulator, as well as novel DNA recognition sites (Table 2). However, it also demonstrates how some binding elements do not fit the standard

TABLE 2. DNA motifs recognized by zinc cluster proteins in *S. cerevisiae*

Zinc cluster	Motif(s) (reference[s])[a]	Cross-regulation/autofeedback loop/ ChIP-chip binding (reference[s])[b]
Arg81p	TGACTCY (162)	Arg81p (92)
Aro80p	CCGNgRNTWRCCGMSAKTTGCCG (162)	Aro80p (92)
Cat8p	YCCNYTNRKCCG (221)	Ume6p (92)
Cep3p	Binds to the CDEIII element of centromeric DNA (63)	
Cha4p	tGCGAtgaR (162)	
Dal81p	GAAAATTGCGTTT (271), AAAAGCCGCGGGCGGGATT (162)	Uga3p (92)
Ecm22p	TCGTATA (274)	
Gal4p	CGG-N_{11}-CCG (272)	
Hap1p	CGG-N_6-CGG (294), CGG-N_3-TANCGG-N_3-TA (89)	Hap1p (92, 104)
Leu3p	CCGG-N_2-CCGG (98), CCGGTMCCGG (162)	
Lys14p	TCCRNYGGA (16)	
Mal63p	MGC-N_9-MGS (244)	
Oaf1p	CGG-N_3-TNRN$_{8–12}$CCG (223)	
Pdr1p	TCCGCGGA (120, 165)	
Pdr3p	TCCGCGGA (51, 98, 120, 165)	Pdr1p, Pdr3p (51, 92, 138)
Pdr8p	TCCG(A/T/C)GGA (100)	
Pip2p	CGG-N_3-TNRN$_{8–12}$CCG (223)	Oaf1p, Pip2p (223)
Ppr1p	TTCGG-N_6-CCGAA (153)	
Put3p	CGG-N_{10}-CCG (242), CGGGAAGCCM-N_3-c (162)	Stb4p (92)
Rds1p	KCGGCCGa (92)	
Rgt1p	CGGANNA (123), SYCGGAAAAA (162)	
Sip4p	TCCATTSRTCCGR (221), CCRTYCRTCCG (276), CGGNYNAATGGRR (92)	Cat8p (276), Ume6p (92)
Stb4p	TCGg-N_2-CGA (92)	Hal9p (92)
Stb5p	CGGNStTAta (92), CGGNSNTA (138)	Stb5p (92, 138)
Sut1p	CGCG (215), GCSGSG-N_2-SG (92), gCSGgg (162)	Sut1p (92)
Tbs1p		Oaf1p, Pip2p (92)
Tea1p	CGG-N_{10}-CCG (86)	
Thi2p	GMAAcYNTWAgA (92), GMAACYSWWAGARCY (162)	
Uga3p	AAAARCCGCSGGCGGSAWT (255), CCGCSSGCGG (195), SGCGGNWttt (107)	
Ume6p	TCGGCGGCT (285), taGCCGCCSa (92)	Oaf1p, Pip2p, Cat8p, Sip4p (92)
Upc2p	TCGTATA (274)	Upc2p (2)
War1p	CGG-N_{23}-CCG (131)	
Ydr520Cp	tCtCCGGCGga (162)	
Yjl103Cp		Ume6p (92)
Yrr1p	WCCGYKKWW (144), TttTGTTACSCR (162)	Pdr1p, Pdr3p, Yrm1p, Yrr1p (158, 296)

[a] S = C or G, W = A or T, R = A or G, Y = C or T, K = G or T, M = A or C, N = A, C, G, or T. Lowercase letters indicate a weaker preference.
[b] The *P* value cutoff for ChIP-chip data from reference 92 was 0.001.

model for zinc cluster proteins, implying that other factors determining DNA-binding specificity can go undetected and have not yet been elucidated.

Mechanisms of Action

The Gal4p superfamily encompasses a wide variety of pivotal, albeit individualized, roles within the cell, and they employ a range of mechanisms in order to do so. Like for many transcriptional regulators, a multitude of strategies exists in order to control their transcriptional activity. These can include nuclear-cytoplasmic shuffling, DNA binding, phosphorylation, and unmasking of the activation domain (236, 249). This section describes some of the known mechanisms in which zinc cluster proteins are transported, activated, aided, or coordinated in order to perform their specific tasks.

Although zinc cluster protein homodimers were once perceived as the "norm" in regulating target genes, recent work demonstrates that many proteins within this class are found predominantly as monomers or heterodimers under physiological conditions. A classic example of a monomer is the *Aspergillus* AlcR protein. NMR spectroscopy clearly shows that one monomeric zinc cluster binds to an element in the *alcA* promoter, which contains the sequence CGTGCGGATC (28). Monomer or dimer status can sometimes be inferred based on the sequence of its target regulatory element. It is proposed that Rgt1p most likely acts as a monomer because its target sequence contains a single trinucleotide, CGGANNA (123). Using these two examples, other zinc cluster proteins that could also potentially regulate target genes as monomers include the *S. cerevisiae* proteins Upc2p and Ecm22p, as well as their homologue Upc2p in *Candida albicans*. They activate transcription of *ERG* genes, which encode enzymes needed for ergosterol biosynthesis, acting through DNA response elements that contain the consensus sequence CGTATA (163, 274). A peculiar exception is Ume6p. Its zinc cluster is localized at the C terminus, and no coiled-coil dimerization region is predicted (233, 260). It was postulated that Ume6p acted as a monomer (248), and this was confirmed when its NMR structure was resolved (7). However, a close examination of its preferred binding sites shows that they actually include two perfect CGG triplets in inverted or direct-repeat orientations. Clearly, NMR spectroscopy and crystal-

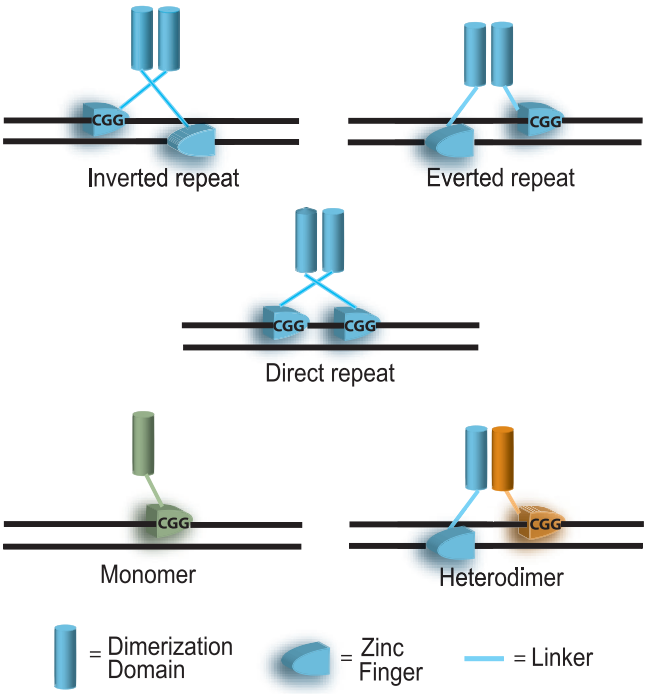

FIG. 3. A model for zinc cluster protein DNA recognition. Zinc cluster proteins preferentially bind to CGG triplets that can be oriented in three different configurations: the inverted, everted, and direct repeats. The orientation of CGG triplets and the nucleotide spacing between the triplets are the two major determinants of DNA-binding specificity (166). Zinc cluster proteins can also bind as monomers (in green) as well as homodimers (two molecules in blue) and heterodimers (one molecule in blue and one in orange).

lography are currently some of the only methods that can determine for certain the dimerization status of members within this protein class.

Several zinc cluster proteins within the pleiotropic drug resistance (PDR) network are able to heterodimerize (see below) (3, 167), although how these heterodimers differentially regulate genes is still unknown. It has long been known that zinc cluster proteins Oaf1p and Pip2p differentially regulate genes by forming heterodimers (115, 117, 223). They regulate genes involved in peroxisome proliferation by acting through oleate response elements (OREs) in the promoters of their target genes *FOX1*, *FOX3*, and *CTA1* (101, 115, 117). In vitro binding assays performed by Rottensteiner et al. (223) show that the Oaf1p/Pip2p heterodimer better binds to the *FOX1* ORE than to the *FOX3* ORE. They concluded that specific sequence differences within the ORE, as well as homodimeric or heterodimeric complexes, must influence promoter recognition (223).

While an Oaf1p homodimer maintains basal levels of target genes, an Oaf1p/Pip2p heterodimer complex is preferred in the upregulation of genes when cells are grown using oleate as a carbon source (115, 117, 223). Zinc cluster proteins can also form heterodimers with members of other transcription factor families, such as members of the MADS box family. Arg81p (ArgRIIp) dimerizes with MADS box proteins ArgRIp and Mcm1p in order to regulate genes that encode enzymes implicated in arginine metabolism (6). These three proteins all possess DBDs, but they must form the three-component complex in order to bind to DNA in vitro in an arginine-dependent manner (6). Amar et al. also suggest that Arg81p acts as the arginine sensor in this complex, because two regions directly N terminal to the DBD share sequence homologies with an arginine-binding pocket in the *Escherichia coli* ArgR repressor (6).

Zinc cluster proteins can further coordinate the transcriptional control of target genes alone or in coordinated networks with other members of this class. They can do so by acting through one or more DNA recognition sites. For example, at least three zinc cluster proteins (Pdr1p, Pdr3p, and Rdr1p) regulate the *PDR5* gene (encoding an ATP-binding cassette [ABC] transporter involved in PDR) by acting through the same pleiotropic drug response elements (PDREs). Conversely, Rgt1p acts alone but requires multiple sites within the promoters of hexose transport genes (123). Another scenario depicts one gene's promoter being regulated by at least two different zinc cluster proteins, acting through two different and exclusive recognition sites. Such is the case for several β-oxidation genes. Ume6p represses the transcription of the genes *CTA1*, *POX1*, *FOX2*, and *FOX3* by acting through a URS1 element, while the Oaf1p/Pip2p heterodimer positively regulates the same genes through OREs in the same promoters (234).

Self-Regulation and Positive Feedback Loops

Several members of this class regulate the expression of other zinc cluster proteins (Table 2). Others are self-regulated, forming a positive feedback loop. In response to oleate, the Oaf1p/Pip2p heterodimer has an additional role in self-activating the *PIP2* gene through another ORE in its own promoter (223). Yrr1p not only is regulated by another zinc cluster protein, Yrm1p (158), but also forms part of an autoregulatory feedback loop. It has been detected at its own promoter in ChIP assays (296). Similarly, Pdr3p is positively autoregulated, as well as being regulated by the Pdr1p zinc cluster protein. Pdr3p controls its own transcription through two PDREs in its promoter (51) and is described in greater detail below. Two other zinc cluster proteins involved in gluconeogenesis, Cat8p and Sip4p, are in this category. Evidence suggests that they regulate themselves by using a complex autoregulatory pathway involving cross talk between the two activators (99, 276). Hap1p is another zinc cluster protein that falls under this umbrella. It regulates genes involved in respiration (134, 295), but its own activity is in part autoregulated (104). ChIP-chip experiments also demonstrate that Stb5p is bound to is own promoter (92, 138). Lastly, studies show that the activator of *ERG* genes, Upc2p, and its *Candida albicans* homologue appear to be involved in positive autoregulation loops (2, 163, 243).

Nuclear Import of Zinc Cluster Proteins and Localization

In order to carry out their functions as transcriptional regulators, members within the Gal4p superfamily must first be localized to the nucleus. Thus, zinc cluster proteins can be categorized based on their initial location within the cell, prior to activating or repressing transcription of their target genes.

The first group consists of those that are permanently present in the nucleus, while the second group resides in the cytoplasm.

Many zinc cluster proteins that make up the first group are constitutively localized within the nucleus on a permanent basis. These include Lys14p, Oaf1p, War1p, Put3p, and Leu3p (11, 59, 126, 127, 131, 234). It has been demonstrated that Oaf1p, War1p, Put3p, and Leu3p are constitutively bound to their target promoters. Put3p is an activator of *PUT* (proline utilization) genes that encode enzymes required for proline metabolism. Although it is always bound to a promoter, its activity is controlled by direct interaction with proline (236, 237). Similarly, Leu3p is bound to the promoters of leucine biosynthesis genes (*LEU4*, *ILV2*, and *ILV5*) but is activated only when a leucine precursor, α-isopropylmalate, is present (126, 254). In addition, it is proposed that Ppr1p, an activator of genes in the pyrimidine biosynthetic pathway, is bound to its target promoters in an inactive state until it is activated by a metabolic intermediate or effector molecule (75). It is postulated that many constitutively active zinc cluster proteins, or "condition-invariant" regulators such as the examples listed above, although not yet identified, must be controlled in this manner (92).

Zinc cluster proteins taking part in transcriptional regulation but initially localized in the cytoplasm must somehow be imported into the nucleus. However, the mechanisms by which they do so are just starting to be clarified. Nuclear import of transcription factors in eukaryotes includes many exclusive pathways. In general, transport of proteins across the nuclear membrane is mediated through nuclear pores wherein soluble transport receptors bind to nuclear localization signals (NLSs) on their target molecules (84, 85, 187). NLSs usually consist of one or two short stretches of basic amino acid residues (reviewed in reference 187). In general, proteins to be shuffled into the nucleus are bound by the α/β importin heterodimer. The α subunit acts as the bridge between the NLS-containing cargo protein and the β subunit, which carries the cargo through the nuclear pore.

Most nuclear import is orchestrated by the importin β receptor family (84), but many nonclassical NLSs on target molecules requiring less conventional nuclear import pathways are also reported (192). Many different importin β or importin-β-like proteins have been characterized in mammalian and yeast cells. Although no general strategy for the import of zinc cluster proteins has been deciphered, a few NLSs have been identified in *Aspergillus* PrnA and AlcR, as well as in *S. cerevisiae* Gal4p and Pdr1p. Gal4p interacts directly (without the help of the α subunit) with the importin β receptor yeast homologue Rsl1p/Kap95p complex, as well as with another importin β called Nmd5p (32, 33). Pdr1p uses the Pse1p/Kap121p complex, which is another member of the yeast importin β-related family (52). AlcR requires three importin β-related proteins: Kap104p, Sxm1p, and Nmd5p. In addition, the NLSs of these aforementioned proteins are located in the N terminus, within or very close to the DBD (192). Thus, differences in nuclear import for a few zinc cluster proteins reflect the many different mechanisms required to fulfill this task, as in higher eukaryotes.

A large-scale protein localization project performed by Huh et al. has provided much insight into zinc cluster proteins and others with respect to their location within the cellular environment (106). Their findings, as well as those of other studies based on the localization of zinc cluster proteins, are summarized in Table 3. The locations of other characterized proteins are assumed based on their functions; Cep3p forms part of the kinetochore complex and should therefore be localized to microtubules and the centromere (106, 143, 250), whereas Rsc3p and Rsc30p share a chromatin-remodeling function and most likely also carry out their roles solely in the nucleus (8).

Activation by Phosphorylation

Several zinc cluster proteins are activated by a phosphorylation or dephosphorylation event. For instance, Gal4p is activated upon phosphorylation. In the absence of galactose, Gal80p represses Gal4p activity by covering its activation domain (161; reviewed in references 236 and 263). Gal4p is phosphorylated at multiple sites (184, 185, 228, 229). Under noninducing conditions, an unphosphorylated form and a phosphorylated form of Gal4p are observed. The presence of the inducer galactose results in the appearance of a second phosphorylated form associated with transcriptionally active Gal4p. Phosphorylation at only a single serine residue (Ser699) in the C-terminal activation domain appears to be necessary for activation (228). However, phosphorylation of Ser699 is not absolutely required for Gal4p activity, since a Ser699-Ala Gal4p mutant shows transcriptional activity in cells lacking Gal80p or in the presence of high galactose levels (219). From these observations, Rohde et al. (219) suggested a model in which phosphorylation of Gal4p is required for an acute response to galactose.

Two other zinc cluster proteins, Pdr1p and Pdr3p, have also been identified as phosphoproteins, although the distinct roles carried out by the phosphorylated isoforms have not yet been elucidated (167). Mamnun et al. eliminated several possibilities for the C-terminally phosphorylated form of Pdr3p, including nuclear localization, dimer formation, and proteolytic turnover (167). In addition, Cat8p and Sip4p are two other members of this family that are characterized phosphoproteins and are activators of genes involved in gluconeogenesis. Both become phosphorylated during the derepression of target genes (34, 212, 276). Likewise, Rgt1p's DNA-binding ability is also regulated by phosphorylation. When cells are grown in glucose, Rgt1p is phosphorylated. This inhibits its binding to its target promoters, thereby preventing its transcriptional repression of hexose transporter genes (74, 123, 182).

At least two zinc cluster proteins are phosphorylated in response to an external stress. War1p is responsible for the upregulation of the gene encoding the ABC transporter Pdr12p in response to weak acid stress. Data suggest that War1p is rapidly phosphorylated in the presence of sorbate, benzoate, and propionate, most likely in order to activate transcription (131). Similarly, Put3p is differentially phosphorylated in the cells' response to different nitrogen sources (105).

Promoter Occupancy

The promoter occupancy of some zinc cluster proteins is influenced by external factors leading to variation in binding at target sites. For example, binding of Upc2p and Ecm22p, two regulators of ergosterol biosynthesis genes, is influenced by

TABLE 3. Classification of zinc cluster proteins in *S. cerevisiae*[a]

Role	Gene name	Localization (reference[s])[b]	Function (reference[s])
Sugar metabolism	*GAL4*	C, N (32)	Activates genes involved in galactose metabolism (*GAL1*, *GAL10*) (24, 168)
	RGT1	C, N (235)	Activator/repressor of hexose transport genes (198)
	MAL13	U	Part of *MAL1* complex locus (35, 189)
	MAL33	U	Activator of maltose genes in maltose metabolism, forms part of *MAL3* complex locus (35, 189)
	MAL63	U	Activator of maltose genes (35, 189)
Amino acid, vitamin, and uracil metabolism	*ARO80*	N	Activator of aromatic acid catabolic genes (108)
	LEU3	N (126)	Activator/repressor of leucine biosynthesis genes (76, 127, 297)
	LYS14	C, N	Activator of lysine metabolic enzymes (68, 69)
	PUT3	C, N	Induction of proline utilization genes (11, 242)
	THI2 (*PHO6*)	U	Activator of thiamine biosynthetic genes (194)
	ARG81	C, N	Activator/repressor of arginine metabolism enzymes (210)
	CHA4	C, N	Induction and basal expression of serine and threonine utilization, activates *CHA1* (102)
	PPR1	N (75)	Activates *URA1* and *URA3* (157)
Miscellaneous	*SEF1*	U	Compensates for the essential function of *RPM2* in cell growth (87)
	TEA1	C, N	Activates transcription of Ty1 retrotransposon (86)
	STB4	N	Interacts with Sin3p in yeast two-hybrid system (118)
Chromatin remodeling	*RSC3*	N	Essential component of the RSC chromatin-remodeling complex (8)
	RSC30	N	Subunit of the RSC chromatin-remodeling complex (8)
Meiosis and mitosis	*UME6*	N	Represses early meiotic genes (7, 202, 248)
	CEP3	M	Essential kinetochore component, chromosome segregation (143, 250)
Nitrogen utilization	*UGA3*	N	Activates GABA genes (277)
	DAL81	N	Activator of nitrogen catabolic genes, including allantoin and GABA genes (25, 40, 110, 277)
PDR/stress response	*PDR1*	N (52, 90)	Activator of PDR genes (15)
	PDR3	N (167)	Activator of PDR genes (53)
	PDR8	C, N	Involved in PDR (100)
	YRM1	C, N	Activator of PDR genes (158)
	YRR1	C, N	Activator of PDR genes (46)
	HAL9	C, N	Involved in salt tolerance (178)
	STB5	C, N	Interacts with Sin3p, is an activator of PDR genes, and is involved in oxidative stress resistance (4, 118, 138)
	RDR1	U	Repressor of PDR genes (97)
	RDS1	U	Regulator of drug sensitivity (4)
	RDS2	U	Regulator of drug sensitivity (4)
	WAR1	N (131)	Activator of *PDR12* in response to weak acid stress (131)
	ASG1 (*YIL130W*)	N	*A*ctivator of *s*tress response *g*enes (C. Wai and B. Turcotte, unpublished data)
Peroxisome proliferation	*OAF1*	C, N (116)	Activates genes involved in peroxisome proliferation (223)
	PIP2	C, N (116)	Activates genes involved in peroxisome proliferation (222)
Ergosterol biosynthesis or uptake	*UPC2*	C, N	Anaerobic sterol uptake, activator of ergosterol biosynthetic genes (44)
	ECM22	C, N	Activator of ergosterol biosynthetic genes (160, 274)
	SUT1 (*YGL162W*)	N (190)	Overexpression increases sterol uptake (190)
	SUT2	U	Overexpression increases sterol uptake (190), multicopy suppressor of low activity of the cyclic AMP/proteinase kinase A pathway (226)
Gluconeogenesis and respiration	*CAT8*	C, N	Activates genes needed for gluconeogenesis (96)
	SIP4	U	Snf1 kinase-dependent activator of gluconeogenesis genes (276)
	HAP1	U	Activates respiration genes (43, 205)
Unknown	*EDS1* (*YBR033W*)	U	Expression is dependent on Rpb2p (S. Vidan and M. Snyder, unpublished data)
	TBS1 (*YBR150C*)	C, N	Δ*ybr150c* is sensitive to thiabendazole (62)
	YBR239C	C, N	Interacts with Rds2p in yeast two-hybrid system (75, 95, 109)
	YDR520C	C, N	Δ*ydr520c* is slightly sensitive to caffeine (5)
	YER184C	U	
	YFL052W	U	Δ*yfl052w* is hypersensitive to heat shock at 37°C (5)
	YJL103C	U	May be involved in oxidative phosphorylation (55)
	YJL206C	U	
	YKL222C	U	Δ*ykl222c* is sensitive to caffeine (5)
	YKR064W	C, N	
	YLL054C	C	
	YLR278C	N	Δ*ylr278c* is sensitive to caffeine (5)
	YNR063W	U	

[a] Known and putative zinc cluster proteins containing the consensus sequence Cys-X_2-Cys-X_6-Cys-X_{5-12}-Cys-X_2-Cys-X_{6-8}-Cys are listed, as well as two other proteins (Sut1p and Sut2p) with divergent cysteine-rich domains. Sut1p has 68 amino acids between the third and fourth cysteines and 17 amino acids between the fifth and sixth cysteines, while Sut2p has 62 amino acids between the third and fourth cysteines. It is not known if these two proteins require zinc for function. Rds3p was initially classified as a zinc cluster protein (4, 5) since it contains the consensus sequence. However, this may be due to the fact that this short protein is cysteine rich (13 cysteines out of 107 amino acids) and not because it is a bona fide zinc cluster protein. Unlike all other zinc cluster proteins, Rds3p has clear orthologues in higher eukaryotes; *S. cerevisiae* Rds3p was shown to be part of the spliceosome (275, 280).

[b] Unless otherwise indicated, localization data are taken from a large-scale study performed by Huh et al. (106). N, nucleus; C, cytoplasm; M, microtubules; U, unknown.

treatment with lovastatin, an inhibitor of the ergosterol pathway. While the level of Upc2p is increased at *ERG3*, the level of the closely related zinc cluster protein Ecm22p is reduced at this same promoter (48). Similarly, genome-wide location analysis (ChIP-chip) revealed that binding of Stb5p is enhanced at some target genes when cells are treated with the oxidative agent diamide. This treatment also leads to Stb5p binding at additional target genes unoccupied by Stb5p in untreated cells (138).

Recruitment of Chromatin Remodelers, Histone Modifications, and Cofactors

Eukaryotic DNA is tightly packaged into chromatin, hampering transcription by limiting DNA accessibility to transcriptional activators and other factors making up the transcriptional machinery. Zinc cluster proteins sometimes require the aid of chromatin-remodeling complexes, histone-modifying enzymes, and/or transcriptional cofactors in order to surmount the repressive nature of chromatin and facilitate gene transcription. This section describes some of the known relationships between zinc cluster proteins and the chromatin remodelers/cofactors that they recruit at their target promoters.

A well-characterized histone acetyltransferase in *S. cerevisiae* is the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex. It is highly conserved throughout evolution (P/CAF complex in humans) (125). Spt and Ada proteins have separate functions apart from the acetyltransferase activity of the Gcn5p subunit (125). A number of yeast genes, including *GAL1-10* and *PDR5*, are SAGA dependent (19, 77, 139, 170). The Gal4p activation domain is responsible for recruiting the SAGA complex to the *GAL1-10* promoter, although transcription is not dependent on the Gcn5p subunit (139).

As described previously, at least three zinc cluster proteins (Pdr1p, Pdr3p, and Rdr1p) regulate the transcription of *PDR5* via PDREs dispersed throughout the promoter (3, 4, 97, 119, 120, 125). An interaction between Pdr1p and the SAGA complex was detected via a two-hybrid assay several years ago, and evidence suggested that perhaps this interaction actually caused an inhibitory effect on *PDR5* expression (170). It has since been clarified that SAGA is needed to actually activate transcription of *PDR5* (77). Spt3p and Spt20p/Ada5p subunits, but not Gcn5p, are needed to activate transcription (77). The *PDR5* promoter is also occupied by other coactivators, including the mediator complex and the chromatin-remodeling SWI/SNF complex (77).

Many yeast genes are negatively regulated by histone deacetylases (HDACs). At least six HDACs exist in yeast. The *RPD3*, *HDA1*, *HOS1*, *HOS2*, *HOS3*, and *SIR2* yeast genes encode HDACs (18). A well-characterized HDAC complex in yeast is the Rpd3p/Sin3p complex. The Rpd3p component exerts the histone deacetylase activity (259), while Sin3p is characterized as a corepressor that depends on Rpd3p to coordinate its repressive effect (114, 279). The Rpd3/Sin3p HDAC complex negatively regulates a variety of genes implicated in numerous cellular processes. Kadosh and Struhl (114) emonstrated that the Ume6p zinc cluster protein relies on this histone-modifying complex in order to repress its target genes. In addition, they showed that only a small region within the Ume6p protein is necessary to recruit this complex to a specific promoter (114). Interestingly, two additional zinc cluster proteins, encoded by the *STB4* and *STB5* genes, interact with the Sin3p corepressor in a two-hybrid assay (118), although a relationship between these interactions and inhibitory transcriptional activity has not been established. Rgt1p is yet another zinc cluster protein that depends on a corepressor in order to exert its repressive effect. It interacts physically with the corepressor Ssn6p in order to negatively regulate *HXT* genes when glucose sources are depleted (197, 208). The Ssn6p-Tup1p complex was first characterized as a general repressor of transcription in yeast (121). Since then, its functional association with multiple HDACs has been elucidated (47, 281, 291).

As their names imply, the ATP-dependent chromatin-remodeling complexes require ATP hydrolysis in order to carry out their chromatin-disrupting function. They are typically composed of several protein subunits. A genome-wide study has revealed that approximately 5% of all yeast genes are SWI/SNF dependent (103). At least two zinc cluster proteins need the SWI/SNF complex at target promoters. Côté et al. showed that Gal4p binding is facilitated and stimulated by the SWI/SNF complex (42). Targeting of SWI/SNF to *GAL1* following galactose induction required the presence of Gal4p (146). Hap1p (an activator of respiration genes, including *CYC1* and *CYC7*) also relies on a functional SWI/SNF chromatin remodeler for transcriptional activity (88).

Other ATP-dependent chromatin remodelers in yeast include the ISWI (*i*mitation *swi*tch)-based family and the RSC (*r*emodels the *s*tructure of *c*hromatin) complex. ISWI complexes are known to organize or displace nucleosomes by sliding them along a stretch of DNA, and this can lead to either repression or activation of target genes (125). The RSC complex is similar to the SWI/SNF complex in that it also contains a large number of subunits. Ume6p is yet another example of a zinc cluster protein that recruits the Isw2p subunit to carry out repression of its target genes, while cooperating with the HDAC complex mentioned earlier (66, 81). It has also been demonstrated that transcriptional activation by Gal4p fusion proteins requires members of the ISW-based family (148, 180). As stated above, the *RSC3* and *RSC30* genes encode zinc cluster proteins that form part of the RSC megacomplex. The zinc clusters of both proteins are needed for proper protein complex function (8). Whether or not they interact directly with DNA by binding to a consensus sequence or whether their DBD motif helps target the complex to RSC-dependent promoters has yet to be elucidated.

ZINC CLUSTER PROTEINS IN *SACCHAROMYCES CEREVISIAE*

The study of zinc cluster proteins in budding yeast has provided much of the framework for understanding fungal transcriptional regulators and their functions within the cell. Sequencing of the *Saccharomyces cerevisiae* genome has allowed for the identification of 55 members within this family (5, 233, 260, 267), based on the well-conserved consensus amino acid sequence of the $Zn(II)_2Cys_6$ motif. This makes it one of the largest families of transcription factors in yeast. Moreover, they can act as repressors, as activators, or as both activators and repressors for certain genes (267). For instance, Rgt1p and Ume6p are both activators and repressors of glucose transport

and early meiotic genes, respectively (110, 198). It has also been recently demonstrated that Stb5p acts as an activator and a repressor in the presence of oxidative stress (138).

Roles

A plethora of cellular processes is orchestrated by members of the Gal4p superfamily. These processes include sugar metabolism, gluconeogenesis and respiration, amino acid metabolism and vitamin synthesis, mitosis, meiosis, chromatin remodeling, nitrogen utilization, and peroxisome proliferation, as well as the stress response and PDR (see below). Table 3 classifies their initially characterized functions into several broad categories and is a compilation of several works (106, 156, 194, 260, 267). Many of these transcriptional regulators not only have more than one distinct role but can also have overlapping functions. They often coordinate gene regulation of different subsets of genes together or at different times. For instance, Ume6p plays a role in nonfermentative metabolism (234), but its primary roles seem to be regulation of early meiotic genes as well as repressing expression of arginine biosynthesis enzymes (7, 22, 110, 125, 202, 224, 248). Another example is Upc2p, whose primary function is in activating ergosterol biosynthesis genes but which also plays secondary roles in anaerobic sterol uptake and expression of *DAN*/*TIR* mannoprotein genes (2, 38, 284). Similarly, zinc cluster proteins Pdr1p and Pdr3p are known for their primary roles in regulating PDR genes, but they also regulate hexose transport genes *HXT9* and *HXT11*, as well as recently being implicated in the transcriptional control of sphingolipid biosynthesis genes (91, 129). Moreover, Pdr3p has other functions that do not include Pdr1p, such as retrograde signaling, as well as a novel role in controlling DNA damage-inducible genes *MAG1* and *DDI1* (298).

Two zinc cluster proteins are essential. Cep3p is part of the kinetochore complex needed during mitosis (143, 250), and Rsc3p is a subunit within the SWI/SNF-like chromatin remodeler RSC. RSC is fairly abundant in cells, and is required for the activation of a number of genes (125). Lastly, Stb5p is also essential but only in certain genetic backgrounds (4, 191, 197).

Amino Acid Metabolism

A number of zinc cluster proteins are involved in controlling expression of genes required for amino acid metabolism. For example, Leu3p is involved in regulating synthesis of branched amino acids (for a detailed review, see reference 127). Cha4p controls expression of genes for catabolism of serine and threonine, while the activator Lys14p is specific for lysine synthesis. Aro80p, another zinc cluster protein, controls expression of genes involved in catabolism of aromatic amino acids (tryptophan, phenylalanine, and tyrosine). These amino acids can be metabolized to alcohols (e.g., tryptophol) for use as a nitrogen source. *ARO9* encodes an aromatic aminotransferase involved in the first catabolic step of aromatic amino acids. *ARO9* expression is increased in the presence of aromatic acids and repressed in the presence of a rich nitrogen source such as ammonia (108). Aro80p positively regulates expression of *ARO9* through a DNA element called UAS_{aro} found in its promoter region (108). Diploid yeast cells can switch to an invasive filamentous form when starved for nitrogen (164). Interestingly, some aromatic alcohols, such as tryptophol, promote morphogenesis (36). Expression of *ARO9* and *ARO10* (another key gene for production of aromatic alcohols) is dependent on cell density or low ammonia concentration and is subject to autoregulation by tryptophol, a process that requires Aro80p (36). Thus, the zinc cluster protein Aro80p is part of a quorum-sensing system bridging environmental conditions to morphogenesis (245).

Like many other zinc cluster proteins, Cha4p activates transcription of target genes in a classical way by binding to specific sequences found in their promoter regions. For example, the Cha4p-dependent expression of *CHA1* (encoding an L-serine/L-threonine deaminase) is induced in the presence of serine or threonine for utilization as nitrogen sources. The *CHA1* promoter contains two UAS_{CHA}s that confer serine or threonine induction when placed in front of a heterologous promoter (21, 102). Interestingly, Cha4p also controls expression of the serine biosynthetic gene *SER3* indirectly via *SRG1* (171, 172). The *SRG1* gene, which does not encode a protein, is located just upstream of the *SER3* gene. Expression of *SRG1* causes transcriptional interference resulting in repression of *SER3*. Cha4p binds to the *SRG1* promoter and is activated in the presence of serine, resulting in *SRG1* transcription and, indirectly, in *SER3* repression (172).

Multidrug Resistance

A large proportion of zinc cluster proteins (at least 12 in *S. cerevisiae*) have been implicated in the cell's response to stress and multidrug resistance. Clearly, the fungal cell must rely on this group of regulators to communicate external or internal environmental pressures. Multidrug resistance, or PDR, is a widespread phenomenon that is highly conserved. It is found throughout evolution in organisms ranging from bacteria to humans and is defined as the cell's ability to become resistant to a multitude of structurally and functionally different cytotoxic compounds (113, 183, 230). PDR is caused by the overexpression of membrane-associated protein pumps and, consequently, expulsion of a wide range of molecules, including antimicrobial drugs (246). In bacteria and other microorganisms such as fungi, multidrug resistance is an evolved and evasive mechanism that presents a major obstacle in the prevention of infectious disease. It also poses many problems in food preparation and agricultural industries. In human beings, acquired multidrug resistance in tumor cells hampers effective chemotherapy. Although most drugs are used against human diseases (cancer) or pathogenic microorganisms (bacteria, protozoans, or fungi), many of the underlying mechanisms in acquired drug tolerance appear to be highly conserved, even among very distantly related organisms (227). Therefore, *Saccharomyces cerevisiae* is an excellent eukaryotic model for providing insight into the phenomenon of pleiotropic resistance.

Many distinct strategies in yeast have been characterized, in relation to how cells respond to different stresses or harmful molecules that can make up an ever-changing cellular environment. They range from very specific regulatory pathways to widespread reactions and are broadly characterized into two interconnected networks: the stress response and the PDR network. Pathways induced in response to stress can buffer

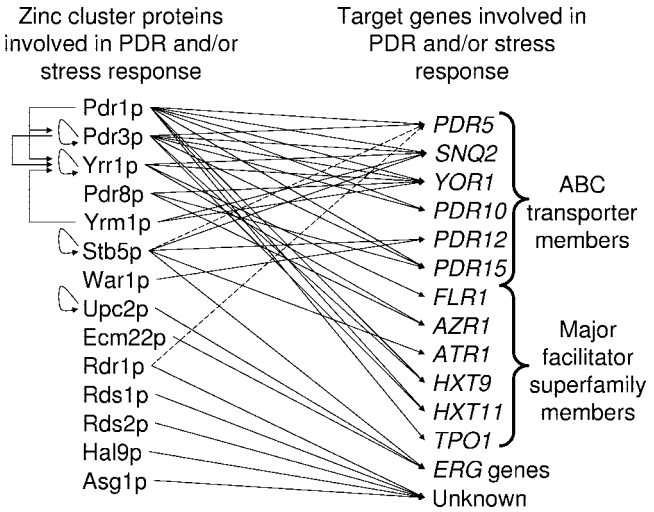

FIG. 4. Zinc cluster proteins involved in the PDR network in *S. cerevisiae*. Zinc cluster proteins (left column) and their target genes involved in PDR and the stress response (right column) are represented. Dashed lines point to genes that are not known to be direct or indirect targets of zinc cluster proteins. Only the most important ABC transporters and major facilitator superfamily members are shown. *ERG* genes are included because they are also involved in drug resistance.

external factors such as heat shock, low pH, weak acids, and high osmolarity (288). As mentioned above, PDR is most often mediated by the upregulation of multidrug efflux pumps or protein transporters, of which there are two types: the ABC transporters and members of the major facilitator superfamily. Two prominent families of transcriptional regulators are equally significant contributing factors in either or both of these networks. They are the bZip protein family (reviewed in reference 183) and the zinc cluster proteins.

Implicated Transcriptional Regulators

Zinc cluster proteins are implicated in PDR because many of them positively regulate the genes that encode drug efflux pumps, thereby conferring drug resistance (Fig. 4). Moreover, drug tolerance and acquired drug resistance in *Saccharomyces cerevisiae* are often traced back to hyperactive or gain-of-function mutations harbored within some of these transcriptional regulators. Pdr1p and Pdr3p are two zinc cluster proteins that have been named the master regulators of drug resistance in budding yeast (reviewed in reference 183). Pdr1p was first characterized by a number of dominant multidrug-resistant alleles that were mapped to its gene's location (213, 232). Pdr3p was initially identified as a gene that conferred resistance to the mitochondrial inhibitor mucidin (252). Together, Pdr1p and Pdr3p are responsible for the regulation, both positive and negative, of multiple genes related to PDR. They act on target genes by binding to PDREs in the promoters of target genes (119, 120, 240). Target genes linked directly to the PDR phenomenon include the ABC transporters encoded by the *PDR5*, *SNQ2*, and *YOR1* genes. The promoters of these genes harbor one or several PDREs. A perfect PDRE regulatory element contains the consensus sequence TCCGCGGA, which displays CGG triplets in an everted repeat orientation. Importantly, the *PDR3* promoter also contains two PDREs, and these elements not only make up a critical component of a positive autoregulatory loop but are also controlled by Pdr1p (51).

As stated above, gain-of-function mutations in Pdr1p and Pdr3p can result in drug resistance due to an increased production of the multidrug efflux pumps. More specifically, at least seven mutations acquired in the *PDR1* gene are considered multidrug resistance mutations. Three of these point mutations (*pdr1-2*, *pdr1-6*, and *pdr1-7*) are within 10 amino acids of each other, located within the structural motifs I and II found in the regulatory domain of Pdr1p, supporting its role as an inhibitory domain. Two other mutations, *pdr1-3* and *pdr1-8*, are found in or just outside the C-terminal activation domain (29). *PDR5* and *SNQ2* mRNA levels are highest in a *pdr1-3* mutant, but they are also elevated in the *pdr1-8* mutant as well. The recently identified *pdr1-12* and *pdr1-33* Pdr1p mutants mediate resistance to the antimicrobial compound diazaborine by overexpressing the ABC transporters Pdr5p, Snq2p, and Ycf1p, as well as the major facilitator superfamily member Flr1p (282). The same study showed that increased mRNA levels of *PDR3* are also caused by the *pdr1-12* allele. Many hyperactive Pdr3p mutants also induce increased expression of *PDR5* and *SNQ2*, as well as *PDR3* (196). Five mutants characterized by Nourani et al. (196) are also located in a short protein segment within structural motifs I and II of the regulatory domain.

The identification of another zinc cluster protein, Yrr1p (*y*east *r*everomycin A *r*esistance), as an additional regulator of PDR genes provided some of the first evidence of cross talk between regulators of PDR in *S. cerevisiae* (296). It also demonstrated that many Pdr1p, Pdr3p, and Yrr1p targets overlap (45, 46). Yrr1p (initially referred to as Pdr2p) was originally implicated in PDR because it bestowed resistance to sulfometuron methyl (an acetolactate synthase inhibitor) (64). It is now known that Yrr1p also confers resistance to the cell cycle inhibitor reveromycin A, to oligomycin, and to 4-nitroquinoline-1-oxide by binding to the *YOR1* promoter and positively regulating the expression of the ABC transporter (45, 64). Efflux of these compounds via Yor1p results in resistance to these toxic compounds. Interestingly, Yrr1p also appears to be self-regulated; it contains a putative Yrr1p response element (YRRE) and a PDRE in its promoter (296). As stated above, expression of *YRR1* is even further regulated by yet another zinc cluster protein, Yrm1p (158). Recent microarray experiments performed by Le Crom et al. provide evidence that Yrr1p positively regulates genes through YRREs containing the consensus sequence T/ACCGC/TG/TG/TA/TA/T (144). It is postulated that Yrr1p targets most likely overlap with Pdr1p/Pdr3p targets because YRRE and PDRE sequences are closely related. A gain-of-function mutant, the *yrr1-1* mutant, provides more insight into how this transcriptional regulator functions. The mutation is a duplication of 12 amino acids located near the C terminus, and it results in a marked resistance to 4-nitroquinoline-1-oxide compared with that of the wild-type strain (46). Northern blot analyses show that *SNQ2* mRNA levels are constitutively elevated in a *yrr1-1* mutant (46).

Other regulators of drug resistance include the zinc cluster proteins Pdr8p, Stb5p, Rds1p, and Rds2p. Pdr8p binds to the promoters of certain genes implicated in PDR, such as *YOR1*,

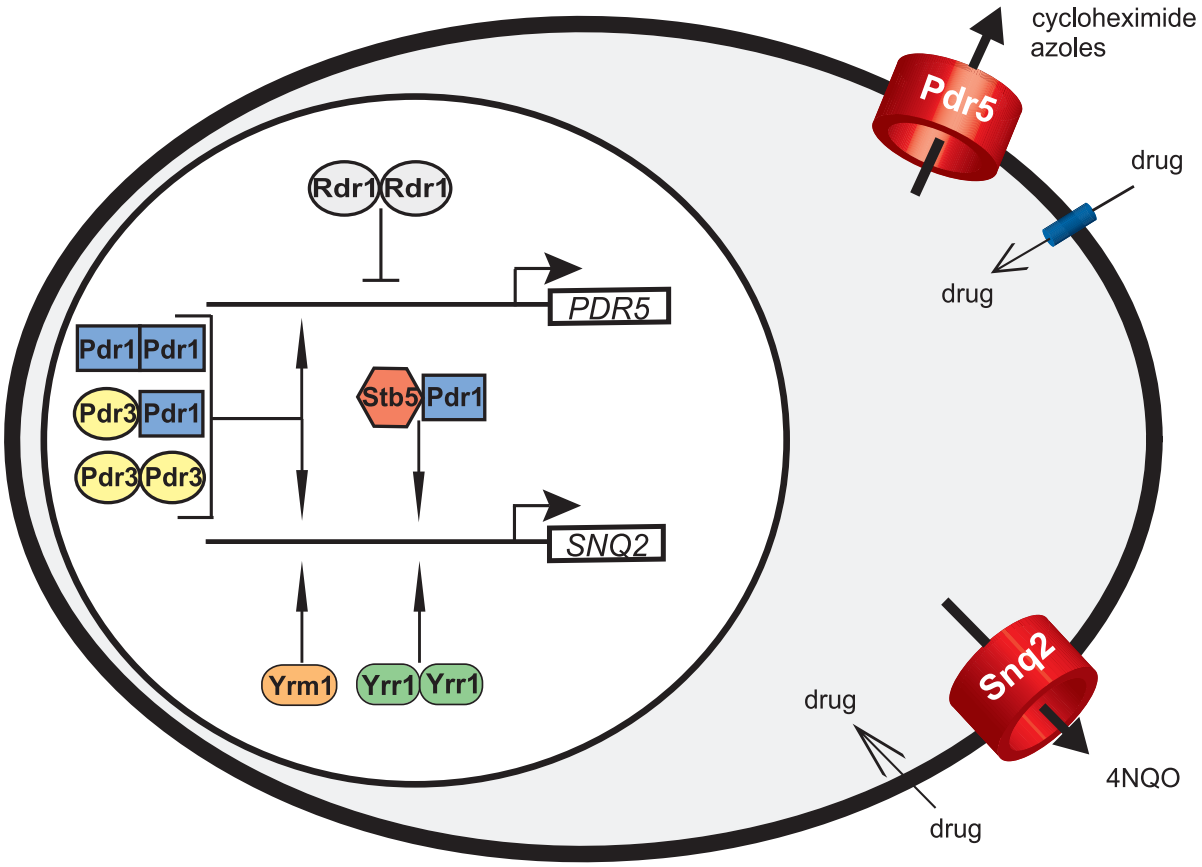

FIG. 5. Interplay among zinc cluster proteins implicated in PDR in budding yeast. A network of characterized zinc cluster proteins cooperatively coordinate the transcriptional regulation of PDR genes in *S. cerevisiae*. Pdr1p (in blue) can form homodimers as well as heterodimers with Pdr3p (in yellow) and Stb5p (in red) (3, 167). Pdr3p, Rdr1p (in gray), and Yrr1p (in green) are able to form homodimers (3, 167; S. MacPherson et al., unpublished data). These different combinations may differentially regulate the expression of *PDR5* and *SNQ2* ABC transporters. A direct binding of Rdr1p to *PDR5* has not been demonstrated, and the mode of binding (e.g., monomer or heterodimer) of Yrm1p (in orange) is not known. 4NQO, 4-nitroquinoline-1-oxide.

PDR15, and *AZR1*. However, this binding was demonstrated using a chimeric Pdr8p; therefore, the exact role of the wild-type protein in PDR is not clear (100). Stb5p was originally picked out of a yeast two-hybrid screen because it interacted with the Sin3p corepressor (118), while Δ*rds1* and Δ*rds2* deletion strains exhibit interesting drug phenotypes that may also implicate them in PDR. Deletion strains Δ*rds1* and Δ*stb5* are hypersensitive to cycloheximide, and a Δ*rds2* deletion strain has severely impaired growth in the presence of the antifungal azole ketoconazole (4). The same study shows that cells lacking *STB5* have reduced mRNA levels of *SNQ2*, *PDR16*, and *PDR5*. Further evidence that Stb5p is a direct positive regulator of *SNQ2* transcription is demonstrated by binding of Stb5p to the *SNQ2* promoter in vivo (138). Moreover, in the presence of diamide, Stb5p is a direct activator of two other genes encoding the drug pumps Atr1p and Pdr12p (138).

Lastly, the Rdr1p (*r*epressor of *d*rug *r*esistance) zinc cluster protein is characterized as a negative regulator of PDR genes (97). A Δ*rdr1* strain is resistant to cycloheximide (4, 97). Rdr1p was confirmed as a transcriptional repressor in microarray experiments that showed that mRNA levels were increased significantly for five genes in the deletion strain compared with the wild-type strain (97). Curiously, all five of these genes (*PDR5*, *PDR15*, *PDR16*, *RSB1*, and *PHO84*) encode membrane or membrane-associated proteins, and four of these genes (with the exception of *PHO84*) actually contain PDREs in their promoters. Furthermore, cycloheximide resistance exhibited in a Δ*rdr1* strain is mediated by the ABC transporter Pdr5p, and Rdr1p appears to act negatively on *PDR5* through the same PDREs used by Pdr1p/Pdr3p to activate transcription (97). Whether or not Rdr1p represses its target genes by binding directly to PDREs has not yet been determined.

The studies mentioned above state that at least three different zinc cluster proteins (Pdr1p, Pdr3p, and Rdr1p) modulate transcription of the *PDR5* gene by acting on the same PDREs (4, 97, 120). Therefore, these three regulators must somehow cooperate together in order to regulate this drug transporter gene. Interestingly, Pdr1p and Pdr3p are capable of heterodimerizing in vivo (167). Stb5p is found predominantly as a Pdr1p/Stb5p heterodimer, while the zinc cluster protein Yrr1p, which regulates *SNQ2*, prefers to form homodimers (3). These interactions describe a complex interplay among regulators of PDR genes (Fig. 5). Moreover, it is hypothesized that Pdr1p acts as the master regulator of drug resistance, because it is the only zinc cluster protein in this network that is able to heterodimerize with more than one partner. It most likely does so

in order to respond to different conditions or changes in the cell's extracellular or intercellular environment, thereby coordinating an effective regulatory pathway (3).

Regulation of Ergosterol Biosynthesis

Yeast can also become resistant to certain drugs by using another strategy, which involves effecting changes in the ergosterol biosynthetic pathway. Several zinc cluster proteins are involved in the regulation of genes in this process. Ergosterol is considered the consensus sterol in fungi because it is the major component of the fungal cell membrane. It performs many crucial roles within the cell, including maintaining membrane fluidity and integrity by generally allowing lipids, membrane-spanning proteins, or membrane-associated proteins to function properly (159). Ergosterol also contributes specifically to the regulation of cell growth and proliferation (206, 211, 217, 218). Many drugs developed to specifically inhibit fungal growth target its biosynthesis (see below).

The ergosterol biosynthetic pathway can be divided into two parts: the biochemical conversion of acetyl coenzyme A into squalene and the transformation of squalene into ergosterol (203). Its synthesis, however, is energetically expensive and oxygen dependent (203). In addition, yeast can accumulate exogenous sterols from the environment only under anaerobic conditions (aerobic sterol exclusion). Therefore, ergosterol biosynthesis, its intermediates, and/or its by-products must perform other critical functions within the yeast cell besides modulating membrane structure (203). The model organism *S. cerevisiae* provides an excellent basis for studying acquired resistance to antifungal drugs, as well as to other toxic compounds. For example, overexpression of the sole azole drug target, a lanosterol 14-α-demethylase encoded by the *ERG11* gene, confers resistance to fluconazole (130).

Upc2p and Ecm22p are two highly homologous zinc cluster proteins in *S. cerevisiae* that regulate expression of *ERG* genes within the ergosterol biosynthetic pathway, including *ERG2* and *ERG3* (274). They positively regulate transcription by acting on sterol response elements in the promoters of their target genes. In fact, at least 11 *ERG* genes encoding enzymes that take part in ergosterol biosynthesis contain putative sterol response elements in their promoters (274). This suggests that regulation by Upc2p/Ecm22p is much more widespread. Upc2p also plays an important role in anaerobic exogenous sterol accumulation, as well as controlling *DAN/TIR* genes that encode mannoproteins involved in anaerobic restructuring of the cell wall (2). Upc2p (*up*take *c*ontrol) was initially characterized by a gain-of-function mutation in the *UPC2* gene that allowed cells to uptake exogenous sterols even when grown in the presence of oxygen (44). An identical mutation in the *ECM22* locus has also been described (241). This *upc2-1* mutant contains a single amino acid change (Gly888Asp) within the activation domain of this protein (44). Interestingly, it was recently demonstrated that the *upc2-1* mutant can upregulate transcription of the ABC transporter genes *AUS1* and *PDR11*, *DAN/TIR* genes, and the *UPC2* gene itself under aerobic conditions (284). This supports previous evidence of another autoregulatory loop within the Gal4p superfamily of zinc cluster proteins (2). It also alludes to a lesser role in the regulation of membrane transporters which may be involved in PDR.

Upc2p and Ecm22p (initially characterized as an *e*xtrac*e*llular *m*utant [160]) are 45% identical according to amino acid sequence and have many overlapping functions (241). More specifically, both zinc cluster proteins have highly similar DBDs and C-terminal activation domains, but their middle regions are quite different (48). It is hypothesized that they must carry out some essential function, as a double-knockout Δ*upc2*Δ*ecm22* strain is nonviable in some backgrounds (241). However, a phenotypic analysis of their deletion strains argues that they must also have distinct roles within the cell that may include PDR. A Δ*upc2* strain is sensitive to the antifungal azole ketoconazole, while a Δ*ecm22* strain is sensitive to cycloheximide (4). Moreover, a recent study shows that Upc2p and Ecm22p respond differently upon induction of the ergosterol biosynthetic pathway by lovastatin. In untreated cells, Ecm22p levels are significantly higher than Upc2p levels (48). Davies et al. (48) showed that in lovastatin-treated cells Upc2p is overexpressed and present in copious amounts at the *ERG3* promoter, while Ecm22p is downregulated and almost nonexistent at the same locus.

Hap1p is another zinc cluster protein that regulates expression of the *ERG11* gene (encodes the azole drug target) in a heme- and oxygen-dependent manner (268, 273). The *HAP1* gene is also upregulated in a *upc2-1* strain, and it is postulated that a regulatory interaction between these two zinc cluster proteins might exist (284). A clear link between Hap1p and drug resistance has not yet been established. Moreover, Stb5p was recently identified as a novel regulator of ergosterol biosynthesis, since it is a direct activator of *ERG5*, *ERG11*, and *ERG25* in the presence of oxidative stress (138).

ZINC CLUSTER PROTEINS IN *CANDIDA ALBICANS*

Candida albicans is typically a commensal organism that inhabits the mucosal linings of most warm-blooded animals, but it is also the major culprit in human fungal infections (231). This fungus was considered asexual for many years, until recent studies proved otherwise (reviewed in reference 17). *C. albicans* is also dimorphic (207). It can switch between a yeast or hyphal mode depending on specific alterations in environmental conditions. These may include changes in temperature and pH or exposure to different compounds such as serum, *N*-acetylglucosamine, or proline (207). The transition into hyphae is implicated in virulence and pathogenesis (142, 154).

From the complete *C. albicans* genome diploid sequence, 77 putative ORFs encode zinc cluster proteins, based on the highly conserved DBD elucidated in *S. cerevisiae* (23). Sequence comparison with known transcriptional regulators in budding yeast reveals many close orthologues. The identification and characterization of zinc cluster proteins in this fungal species has just started, and only a few them have been designated specific functions. Known zinc cluster proteins and their roles within the cell are summarized in Table 4. Functions include sugar metabolism, ergosterol biosynthesis, regulation of hyphal growth, and PDR. One can speculate that as the roles of more zinc cluster proteins in this species are elucidated, many of the proteins will be implicated in a variety of physiological roles similar to those displayed in budding yeast.

TABLE 4. Characterized zinc cluster proteins in *C. albicans*

Gene	orf19	Function
CWT1	orf19.5849	Δ/Δ*cwt1* strain is sensitive to calcofluor white and alters the composition of the cell wall (181)
CZF1	orf19.3127	Hyphal growth regulator (26, 283)
FCR1	orf19.6817	Negative regulator of drug resistance, complements an *S. cerevisiae* Δ*pdr1* Δ*pdr3* strain (256)
FGR17	orf19.5729	Regulator of filamentous growth (269)
FGR27	orf19.6680	Regulator of filamentous growth (269)
SEF2	orf19.1926	*SEF2* expression is repressed by *SFU1* under high-iron conditions (137)
SUC1	orf19.7319	Regulates sucrose metabolic genes (122)
TAC1	orf19.3188	Transcriptional activator of *CDR1* and *CDR2* multidrug transporter genes (41)
UPC2	orf19.391	Transcriptional activator of ergosterol biosynthetic genes (163, 243)
WAR1	orf19.1035	Confers resistance to sorbate (141)
ZNC1	orf19.3187	Encodes an essential protein of unknown function (41)
ZNC3	orf19.3190	Encodes an essential protein of unknown function (41)

Transcriptional Regulators of PDR

A vast number of transcription factors in budding yeast coordinate the control of several genes involved in drug resistance. Since the genetic manipulation and study of *C. albicans* are slow in comparison, only two zinc cluster proteins so far are definitely linked to PDR in this species. *FCR1* encodes a zinc cluster protein that was cloned from a library as a gene that was able to complement a Δ*pdr1* Δ*pdr3* phenotype. Overexpression of *FCR1* (*f*luconazole *r*esistance 1) in budding yeast resulted in increased resistance to fluconazole and cycloheximide, as well as an increase in *PDR5* expression (256). Surprisingly, that study also demonstrated that a *C. albicans* Δ*fcr1*/Δ*fcr1* homozygous deletion strain is actually hyperresistant to fluconazole and other antimycotic drugs. The authors concluded that Fcr1p must be a negative regulator of drug resistance genes. The specific promoters targeted by Fcr1p and its mechanism of action have not yet been identified.

Tac1p (*t*ranscriptional *a*ctivator of *Candida* drug resistance genes) is the second *C. albicans* zinc cluster protein directly associated with the regulation of PDR genes (41). Initially, de Micheli et al. showed that a common drug response element (DRE) found in the *CDR1* (DRE_I) and *CDR2* (DRE_{II}) promoters was responsible for drug-induced upregulation of both of these ABC transporter genes (54). They observed that these DREs might be putative zinc cluster binding sites because they contained CGG triplets in a direct-repeat orientation. A genome-wide search for putative proteins containing the highly conserved $Zn(II)_2Cys_6$ motif mapped the *TAC1* gene to a region near the mating-type locus that was previously linked to azole resistance in a few clinical isolates (225). A heterozygous deletion of the *TAC1* gene caused a loss in *CDR1* and *CDR2* upregulation in response to fluphenazine, while a glutathione *S*-transferase–Tac1 fusion protein can bind these DREs in vitro (41). Furthermore, a *TAC1-2* mutant recovered from an azole-resistant strain is responsible for the constitutive overexpression of both of these ABC transporters (41). This evidence provides substantial proof that Tac1p is a bona fide regulator of multidrug resistance.

Ergosterol Biosynthesis in *C. albicans*

Numerous and extensive studies encompass the ergosterol biosynthetic pathway in this opportunistic pathogen. Only one clear zinc cluster protein orthologue corresponds to both Upc2p and Ecm22p in *Candida albicans* (163, 243). Cells lacking *UPC2* are susceptible to several antifungal compounds that target enzymes within the pathway and cell wall formation (including azoles, terbinafine, fenpropimorph, and lovastatin) (163, 243), while overexpression of the *C. albicans UPC2* gene renders cells resistant to fluconazole, ketoconazole, and fluphenazine (163). The Upc2p orthologue is similarly important for aerobic sterol uptake in *C. albicans*, as demonstrated by a discernible reduction of [^{14}C]cholesterol accumulation in a Δ*upc2* strain (243).

ZINC CLUSTER PROTEINS IN OTHER SPECIES

Zinc cluster proteins are also found in other yeast species, as well as other fungi. These organisms are not as well studied, but many $Zn(II)_2Cys_6$ regulators have been characterized, and these are listed in Table 5. Some of these zinc cluster proteins have clear orthologues in *S. cerevisiae*. For example, the Gal4p regulator of galactose catabolism in *S. cerevisiae* is Lac9p in *Kluyveromyces lactis*. The *LAC9* gene is able to complement a Δ*gal4* strain (290). Furthermore, the regulator of purine utilization in *Aspergillus nidulans*, UaY, is closely related to Ppr1p in *S. cerevisiae* and is able to recognize an identical DNA motif (251).

One of the most well-studied zinc cluster proteins in filamentous fungi is AlcR from *Aspergillus nidulans* and its role in ethanol catabolism (reviewed in reference 67). In brief, the AlcR transcriptional activator is essential (along with the coinducer acetaldehyde) for the utilization of ethanol as a carbon source in this species. It controls expression of the *alcA* and *aldA* genes (133, 200), which encode an alcohol dehydrogenase and an aldehyde dehydrogenase, respectively. These enzymes convert alcohol into acetaldehyde and, secondly, acetaldehyde into acetate. The AlcR activator also undergoes autoregulation (133, 177). In the presence of glucose, transcription of these genes is shut down by binding of the repressor CreA directly to the *alcR* and *alcA* promoters (176, 201). As stated above, AlcR binds as a monomer to inverted, everted, and direct repeats found into the promoters of *alcR*, *alcA*, and *aldA* (see Fig. 2).

One important characteristic of filamentous fungi is the production of a wide range of secondary metabolites. Many of these natural compounds are important in medical and/or agricultural fields. Aflatoxins are secondary metabolites produced by several *Aspergillus* species. These compounds are

TABLE 5. Characterized zinc cluster proteins in other species

Gene	Species	Function
ACEII	*Trichoderma reesei*	Activator involved in regulation of cellulase and xylanase genes (9)
AFLR	*Aspergillus parasiticus*, *Aspergillus nidulans*	Involved in regulation of the aflatoxin pathway (57, 58, 71, 289)
ALCR	*Aspergillus nidulans*	Activator of genes required for ethanol oxidation (133, 193, 200, 238)
AMYR	*Aspergillus nidulans*, *Aspergillus oryzae*, *Aspergillus niger*	Activator involved in amylolytic gene expression (83, 258)
ARCA	*Aspergillus nidulans*	Involved in the arginine catabolic pathway (60)
CLTA1	*Colletotrichum lindemuthianum*	Involved in the switch between biotrophy and necrotrophy during infection (56)
CMR1	*Colletotrichum lagenarium*	Involved in melanin biosynthesis (266)
CRG1	*Cercospora nicotianae*	Involved in cellular resistance to cercosporin (37)
*CTF1*α, *CTF1*β	*Nectria hematococca*	Activator of cutinase genes (150, 151)
FACB	*Aspergillus nidulans*, *Neurospora crassa*	Activator of acetate regulatory genes (20, 261, 262)
FL	*Neurospora crassa*	Required for conidiophore morphogenesis (13, 14, 216)
LAC9	*Kluyveromyces lactis*	Controls induction of the lactose-galactose regulon (147, 286, 290)
MLCR	*Penicillium citrinum*	Involved in ML-236B (compactin) biosynthesis (1)
MOC3	*Schizosaccharomyces pombe*	Involved in sexual development, ascus formation, and stress response (80)
NIRA	*Aspergillus nidulans*	Regulator of nitrate assimilation (27, 188, 209, 247)
NIT4	*Neurospora crassa*	Activator of the nitrate assimilatory pathway (70)
PDR1	*Candida glabrata*	Activator of PDR genes (264)
PIG1	*Magnaporthe grisea*	Involved in melanin biosynthesis (266)
PRNA	*Aspergillus nidulans*	Activator of genes involved in proline utilization (30, 82)
PRO1$^+$	*Sordaria macrospora*	Required for fruiting body development (175)
QUTA	*Aspergillus nidulans*	Regulates expression of genes involved in quinic acid utilization (149)
QUTH	*Aspergillus nidulans*	Possibly involved in the regulation of genes required for utilization of protocatechuic acid (136)
TAMA	*Aspergillus nidulans*	Involved in nitrogen regulation (49)
THI1	*Schizosaccharomyces pombe*	Activator of several thiamine-repressible genes (65, 257)
UAY	*Aspergillus nidulans*	Activator involved in purine utilization and transport (31, 251)
XLNR	*Aspergillus niger*, *Aspergillus oryzae*	Controls expression of genes encoding xylanolytic enzymes (93, 94, 173, 174)
YNA2	*Hansenula polymorpha*	Activator of the genes involved in nitrate assimilation (10)
ZFR1	*Fusarium verticillioides*	Involved in regulation of fumonisin biosynthesis (73)

highly toxic and carcinogenic in animals and humans (292). Indeed, epidemiological studies have established that aflatoxin exposure is a major risk factor for liver cancer (292). Interestingly, the aflatoxin production pathway is regulated by the zinc cluster protein AflR. Genes encoding enzymes of this pathway are located in a 70-kb gene cluster in both the *Aspergillus flavus* and *Aspergillus parasiticus* genomes (292). AflR positively regulates the expression of these genes by binding to the consensus motif TCGSWNNSCGR found in the promoters of target genes (58).

Colletotrichum lagenarium is a plant pathogen that causes anthracnose of cucumbers. Infection of host plants by this fungus requires the production of melanin for successful invasion by formation of an infection structure called the appressorium (132). Cmr1p, a $Zn(II)_2Cys_6$ protein, is a positive regulator of the expression of melanin biosynthetic structural genes *SCD1* and *THR1* in this species (266). *ZFR1* is another example of a gene encoding a zinc cluster protein involved in secondary metabolite production. In the phytopathogenic fungus *Fusarium verticillioides*, the biosynthesis of fumonisin B_1, a mycotoxin that causes leukoencephalomalacia in equids and pulmonary edema in swine (220), is severely impaired in *zfr1* mutants (73). Interestingly, the fungus *Penicillium citrinum* produces ML-263B (compactin), which can act as a substrate for production of pravastatin sodium, a compound used in hypercholesterolemia therapy (1, 239). The zinc cluster protein MlcRp is a *P. citrinum* transcriptional regulator that is important in its production (1). The crucial roles of fungal secondary metabolites in various biological fields imply that many other zinc cluster proteins and their key functions are likely to be further investigated in the near future.

CONCLUSION

Since its isolation in 1982 (112, 140), the *GAL4* gene has become the focus of numerous studies, leading to in-depth knowledge of its mechanism of action. Gal4p was one of the first eukaryotic transcription factors to be characterized, and it is now considered a classical model for eukaryotic transcription. Over the years, many other zinc cluster proteins have been characterized, and as a result, general rules for these transcription factors (and others) can now be derived.

The zinc finger regions of the members of the Gal4p family have a specific structure that is unique to fungi. X-ray and NMR analyses of the DNA-binding domains of some members of the family show that the cysteine-rich region has a remarkably similar structure that commonly recognizes CGG triplets. Residues flanking the cysteine-rich region are responsible for differences in DNA-binding specificity. For example, changing the relative orientation of the two zinc clusters of a homodimer allows recognition of inverted, direct, or everted repeats. In addition, alteration of the relative distance between the two zinc clusters further increases the repertoire of binding sites by allowing binding of a homodimer to CGG triplets with different spacings. Alternate modes of DNA recognition such as monomeric or heterodimeric binding have also been described.

Zinc cluster proteins function in a wide range of processes, including amino acid and vitamin synthesis, carbon and nitro-

gen metabolism, meiosis, and morphogenesis. Other roles include regulation of genes involved in the stress response and pleiotropic drug resistance, as demonstrated in budding yeast and in human fungal pathogens. While Gal4p appears to act solely as an activator, a growing number of zinc cluster proteins have been shown to have both activator and repressor capabilities. Genome-wide studies also show that many zinc cluster proteins have both distinct and overlapping functions. In addition, autoregulation and cross-regulation of the expression of zinc cluster proteins are becoming a theme that is more and more common. Mechanisms that regulate activity of zinc cluster proteins include phosphorylation (e.g., Cat8p and Sip4p), binding of a small inducer molecule to the factor (e.g., binding of proline to Put3p), and interaction with a metabolic intermediate (e.g., Leu3p). With the number of characterized zinc cluster proteins growing rapidly, it is becoming more and more apparent that they are crucial regulators of fungal physiology. Furthermore, their potential importance extends toward infectious diseases and the agricultural industry. From its beginnings as a pioneer model for eukaryotic transcription, the study of this family of transcriptional regulators has clearly evolved and reached a much broader significance.

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