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REVIEW ARTICLE

Transcription factors and ABC transporters: from pleiotropic drug resistance to cellular signaling in yeast

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Budding yeast *Saccharomyces cerevisiae* survives in microenvironments utilizing networks of regulators and ATP-binding cassette (ABC) transporters to circumvent toxins and a variety of drugs. Our understanding of transcriptional regulation of ABC transporters in yeast is mainly derived from the study of multidrug resistance protein networks. Over the past two decades, this research has not only expanded the role of transcriptional regulators in pleiotropic drug resistance (PDR) but evolved to include the role that regulators play in cellular signaling and environmental adaptation. Inspection of the gene networks of the transcriptional regulators and characterization of the ABC transporters has clarified that they also contribute to environmental adaptation by controlling plasma membrane composition, toxic-metal sequestration, and oxidative stress adaptation. Additionally, ABC transporters and their regulators appear to be involved in cellular signaling for adaptation of *S. cerevisiae* populations to nutrient availability. In this review, we summarize the current understanding of the *S. cerevisiae* transcriptional regulatory networks and highlight recent work in other notable fungal organisms, underlining the expansion of the study of these gene networks across the kingdom fungi.

Keywords: ABC transporters; cellular detoxification; cellular signaling; cellular stress; drug resistance; gene regulation; lipid transport; pleiotropic drug resistance; transcription factors; yeast

ATP-binding cassette (ABC) transporters have evolved to play critical roles in a multitude of cellular processes. Throughout the different domains of life, ABC transporters possess a diverse set of regulatory mechanisms including but not limited to auto-inhibition by substrates, post-translational modifications, protein binding, and modulation of protein concentration [1–5]. There is great interest in the ABC transporter field to explore how such transporters are regulated and the impact of this regulation on cellular processes.

The model organism *Saccharomyces cerevisiae* has contributed greatly to our understanding of eukaryotic gene regulation. Its proteome possesses homologs to other systems including both fungal pathogens such as *Candida glabrata* and *Candida albicans* as well as humans, making it an ideal system to explore how gene regulation impacts the function of ABC transporters in eukaryotes. The genome of *S. cerevisiae* contains 22 ABC transporters (Fig. 1), many of which are essential for adaptation to environmental challenges [6]. The

Abbreviations
4-NQO, 4-nitroquinoline-*N*-oxide; ABC transporters, ATP-Binding Cassette transporters; ChIP, chromatin immunoprecipitation; CRD, cysteine-rich domain; EMSA, electrophoretic mobility shift assay; MFS transporters, major facilitator superfamily transporters; NLS, nuclear localization sequence; PDR, pleiotropic drug resistance; PDREs, pleiotropic drug resistance elements; P-gp, P-glycoprotein; PI, phosphatidylinositol; ROS, reactive oxygen species; SRE, sterol response element; STREs, stress response elements; Yap, Yeast activator protein.

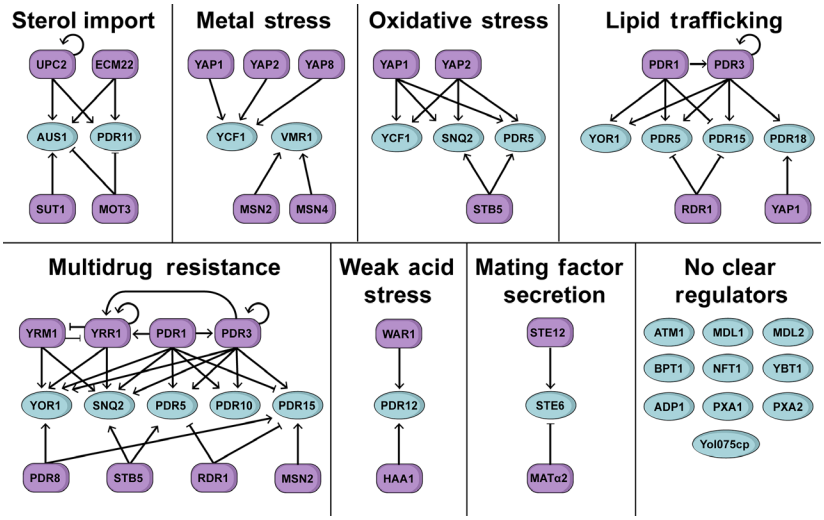

Fig. 1. Gene networks of *S. cerevisiae* transcription factors and ABC transporters. The ABC transporters (blue) and their regulators (purple) are shown in the different cellular processes they participate in: sterol uptake, oxidative stress, metal stress, weak acid stress, multidrug resistance (PDR), lipid trafficking, and mating factor secretion. Note that many yeast ABC transporters have no clear transcriptional regulators. Many of the transcription factors have been shown to regulate other genes with overlapping roles in cellular functions.

majority of the yeast ABC transporters have specific temporal or environmental contexts in which they function, making them frequent targets for transcriptional regulation. For example, the transporter Ste6 exports mating factor necessary for yeast mating, which occurs in only a portion of the yeast cell cycle, while Pdr12 is only necessary in environments abundant in weak acids [7,8]. Ste6, Pdr12, and other yeast ABC transporters have variable expression that peaks in the conditions in which their function is required.

Over the past four decades, numerous transcription factors (TFs) have been identified that regulate the expression of the yeast ABC transporters. These TFs regulate biological processes by binding to promoter regions that control expression of target genes. The characterization of TFs impacting ABC transporter expression has frequently lagged behind that of the transporters, with several remaining partially uncharacterized. However, transcriptional regulation is inextricably intertwined with ABC transporter biology. As our understanding of TFs has evolved, their gene networks have not only solidified the known roles of ABC transporters in cellular functions, but also indicate that they participate in a range of additional roles. Whole-genome duplication of the common ancestor of *S. cerevisiae* and related yeast species created ohnologs of ABC transporters and TFs, allowing these species to rewire their regulatory networks to respond to environmental stressors in new ways. There is also growing evidence that many of these transcriptional regulators are sensors, directly linking the presence of substrate with expression of the respective ABC transporter.

This review provides a comprehensive summary of currently known yeast TFs that regulate ABC transporters, connecting the roles of both classes of proteins in cellular detoxification, sterol import, lipid trafficking, and cellular signaling.

The role of master regulators Pdr1 and Pdr3 in multidrug resistance

Microorganisms frequently compete with each other for resources in the environment, leading to the evolution of a variety of toxins to inhibit their competitors [9]. *S. cerevisiae* has evolved robust resistance to mycotoxins produced by other fungal species [10]. 40 years ago, several *S. cerevisiae* strains were isolated that displayed resistance to a broad range of drugs and natural toxins, referred to as pleiotropic drug resistance (PDR) [11]. Work over the following two decades identified the genes contributing to PDR as primarily ABC transporters, major facilitator superfamily (MFS) transporters, and zinc cluster TFs [12–17]. The best characterized PDR ABC transporters Pdr5, Snq2, and Yor1 each play a role in resistance against hundreds of functionally and structurally unrelated drugs [18]. The most frequent loci mutated in PDR strains was found to encode two zinc cluster TFs Pdr1 and Pdr3, which are paralogs with 36% identity [19,20]. Subsequent work identified that knockouts of either TF resulted in a substantial decrease in drug resistance and the loss of drug induced expression of ABC and MFS transporters, establishing Pdr1 and Pdr3 as an essential part of the PDR network [12,21–23].

Much of our understanding of the function of Pdr1 and Pdr3 is based on the promoter of the ABC transporter *PDR5*, one of the primary drug efflux pumps. Initially, both Pdr1 and Pdr3 were found to bind to 3 sites known as pleiotropic drug resistance elements (PDREs) within the *PDR5* promoter that were

essential for inducibility of the transporter [3,21,24]. Sequencing of the promoters of other PDR ABC transporters identified one or multiple canonical or degenerate PDREs reinforcing the idea that Pdr1 and Pdr3 are direct regulators of the PDR gene network [25,26]. Pdr1 and Pdr3 are constitutively bound to DNA regardless of the presence of drugs, suggesting that activation of both is not dependent on changes in nuclear occupancy or DNA-binding [27,28].

Like other known zinc cluster proteins, partial deletions or mutations within the regulatory domains of Pdr1 and/or Pdr3 results in constitutively active TFs that induce strong expression of ABC transporters [29–31]. For example, the Pdr1 mutants *pdr1-3* and *pdr1-6* result in up to 10-fold higher minimum inhibitory concentrations of cycloheximide, oligomycin, ketoconazole, and 4-nitroquinoline-*N*-oxide (4-NQO) [29]. Work by Thakur *et al*. [32] demonstrated that the regulatory domains of both Pdr1 and Pdr3 bind the antifungal ketoconazole at low micromolar affinity, leading to the speculation that ligand binding may increase transcriptional activity. Characterization of Pdr1 mutants has also proven useful in the yeast membrane biology field as *pdr1-3* has proven an effective tool for overexpression, and hence biochemical study, of Pdr5 and other membrane proteins [33,34].

Pdr1 and Pdr3 form homodimers like most zinc cluster proteins, but also are capable of forming heterodimers. However, it is unclear if the function of heterodimers differs from homodimerization or has any function at all [27]. While Pdr1 and Pdr3 share DNA-binding sites, they appear to have different, but overlapping functions. Pdr1 and Pdr3 both regulate expression of Pdr3 through two PDREs in the *PDR3* promoter creating a positive feedback loop [35]. Gene knockout experiments demonstrated that Pdr1 is required for the early (< 1 h) induction of PDR genes in response to drug exposure, while Pdr3 and other PDR associated TFs are not [36]. On the other hand, Pdr3 but not Pdr1 induces expression of PDR genes when mitochondrial function is disrupted such as loss of the mitochondrial genome [37]. While the mitochondrial signal that activates Pdr3 remains unclear, recent work has identified that Pdr3 responds to mitochondrial protein import stress where Pdr3 induces expression of *CIS1* to promote clearance of unimported proteins that otherwise impairs mitochondrial function [38]. These studies established that Pdr1 plays a major role in the initial drug response, including increasing the expression of Pdr3, while Pdr3 is involved in an additional signaling pathway.

The closely related pathogenic fungi, *Candida glabrata,* possess three drug efflux ABC transporters, *Cg*Cdr1, *Cg*Pdh1, and *Cg*Snq2 regulated by *Cg*Pdr1 [39–44]. *Cg*Pdr1 appears to be a blend of both *Sc*Pdr1 and *Sc*Pdr3 as it shares higher homology with *Sc*Pdr1, but also possesses several characteristics of *Sc*Pdr3 including autoregulation and response to loss of mitochondrial DNA [41,45,46]. Two drug efflux ABC transporters, *Ca*Cdr1 and *Ca*Cdr2 from *Candida albicans* have similar functions to *Sc*Pdr5, but a *C. albicans* Pdr1 homolog is absent [47–49]. Instead, *C. albicans* has evolved a different zinc cluster protein, Tac1, that regulates *Ca*Cdr1 and *Ca*Cdr2 in a homologous fashion to *Sc*Pdr1 [50,51]. Like *Sc*Pdr1, Tac1 is required for strong drug resistance, and many *C. albicans* drug-resistant clinical isolates have mutations that either overexpress Tac1 or alter its function [50–52]. The distally related filamentous fungal pathogen *Aspergillus fumigatus* also lacks a Pdr1 homolog, but has intrinsic drug resistance to azole antifungal drugs through the ABC transporter *abcG1* and its zinc cluster TF regulator, *atrR* [53,54].

The other PDR zinc cluster transcription factors

While Pdr1 and Pdr3 are the primary regulators of the PDR ABC transporters, a number of other zinc cluster TFs also regulate the PDR network. These additional zinc cluster proteins appear to have much narrower functions than either Pdr1 or Pdr3 as they each regulate different subsets of the PDR ABC transporters. Together, these seven TFs are responsible for the expression of five drug efflux ABC transporters, *PDR5*, *PDR10*, *PDR15*, *SNQ2*, and *YOR1* (Fig. 1). In alignment with regulating subsets of the PDR network, these additional zinc cluster proteins each have a narrower, but significant impact on *S. cerevisiae* drug resistance.

The next zinc cluster TF identified after *PDR1* and *PDR3* was *YRR1*, which was first isolated during resistance screening for the cell cycle inhibitor, reveromycin A. Deletion of *YRR1* results in hypersensitivity to oligomycin and 4-NQO but not the *PDR5* substrate cycloheximide [55]. *YRR1* hyperactive mutants such as *yrr1-2* (*pdr2-2*) display increased resistance to oligomycin and 4-NQO similar to what has been observed in *pdr1-3* [56]. The ABC transporters *YOR1* and *SNQ2* confer resistance to oligomycin and 4-NQO, respectively, which implicated their regulation by *YRR1* [57,58]. Further studies utilizing promoter deletions, chromatin immunoprecipitation, and electrophoretic mobility shift assay (EMSA) demonstrated that Yrr1 indeed binds to the promoters of *YOR1* and *SNQ2* [57–59]. *YRR1* appears to be the primary regulator of

SNQ2 as Δ*yrr1* strains have severely reduced *SNQ2* mRNA levels, while Δ*pdr1*Δpdr3 only reduces basal expression by 50% [55]. Like Pdr1/Pdr3, Yrr1 also regulates the MFS transporters *AZR1*, *SNG1* and *FLR1*. Reminiscent of *PDR3* and *YRR1* is regulated by Pdr1/Pdr3 through a PDRE in the *YRR1* promoter, as well as self-regulation through a Yrr1 binding site (Fig. 1) [58]. The consensus DNA binding sequence that was identified for Yrr1 is highly similar to a PDRE, except for three degenerate base pairs [59]. Despite nearly identical DNA binding motifs, Yrr1 was not found to form heterodimers with Pdr1 or Pdr3 [60].

Phylogenetic tree analysis of the zinc cluster family identified *YRR1* as part of a four gene cluster with high similarity consisting of *YRM1*, *PDR8,* and *YKL222C* [59]. *PDR8* is the ohnolog of *YRR1* that arose from whole-genome duplication, while *YRM1* and *YKL222C* are paralogs of *YRR1* that arose from two separate gene duplications. *YRM1* was identified as the closest paralog of *YRR1* with 41% identity. With high sequence conservation in their DNA binding domains, it was predicted that both Yrr1 and Yrm1 share a DNA binding site. *YRM1* was found to regulate 14 out of 15 of the same genes as *YRR1*, as well as an additional 9, revealing that while these proteins regulate similar genes they are not redundant [61]. Yrm1 and Yrr1 exhibit a complex regulatory relationship where either becomes more active when the other is removed from the cell, although Yrm1's increase in activity is more dramatic. In the absence of Yrr1, Yrm1 exhibits highly increased promoter binding and induction of expression. Interestingly, deletion of the Yrm1 regulatory domain results in promoter occupancy with no significant difference between the presence or absence of Yrr1. This would suggest that Yrm1's inhibition by Yrr1 is not due to competitive DNA binding, but rather some interaction with the regulatory domain. While much remains to be unraveled about the function of this pair of TF paralogs, they are reminiscent of Pdr1 and Pdr3 with their closely intertwined functions. The third member of the Yrr1 subfamily, Pdr8, binds to and regulates many of the same genes as Yrr1 and Yrm1, though notably regulates *PDR15*, but not *SNQ2* (Fig. 1) [62]. Consequently, null mutants of *PDR8* display sensitivity to oligomycin-like *YRR1 via* regulation of *YOR1*, but not to *SNQ2* substrates such as 4-NQO. The last remaining member of the Yrr1 subfamily, *YKL222C*, remains uncharacterized but presumably also regulates a portion of the PDR network.

A screen of zinc cluster deletion strains assaying the growth in the presence of various drugs identified two additional proteins that impacted drug resistance, *STB5* and *RDR1* [63]. The deletion of *STB5* provided a significantly reduced resistance to cycloheximide, a Pdr5 substrate. The *Δstb5* strain had reduced levels of *PDR5* and *SNQ2* mRNA, implicating Stb5 as another regulator of these two transporters. An EMSA with the purified Stb5 DNA binding domain demonstrated Stb5 is capable of binding to a PDRE, suggesting that like Pdr1 and Pdr3 it regulates genes through direct binding to PDREs [63]. Co-immunoprecipitation identified that Stb5 interacts with Pdr1, but not Pdr3 *in vivo* [60]. Based on an EMSA of the DNA binding domains of Pdr1 and Stb5, the two can form a heterodimer DNA complex with unknown significance [60]. The other identified TF was *RDR1*, a repressor of drug resistance, that reduces the expression of 5 genes, including the ABC transporters *PDR5* and *PDR15* [64]. This repression of mRNA levels is dependent on the sequences of the PDREs present in the promoter region of 5 genes, suggesting Rdr1 also recognizes PDREs. Further work on Pdr1 and Pdr3 interaction partners found that both interact with Rdr1 *in vivo* [60]. It appears likely that Rdr1 forms heterodimers with Pdr1 and Pdr3 on the PDREs found in the promoters of the 5 repressed genes, but not at other PDREs, suggesting PDRE flanking sequences may play an important role in dimer formation.

While there is still much to unravel about the function of many PDR-associated zinc cluster TFs, what is clear is that they function with Pdr1 and Pdr3 to form a complex network regulating ABC transporters. Not only are four zinc cluster proteins (Pdr1, Pdr3, Stb5, and Rdr1) capable of binding to PDREs, but they also each form different heterodimers with Pdr1. The functional differences between homodimers and heterodimers remain unclear, but heterodimerization may serve to integrate simultaneous activation of these TF combinations into a specific response. The most recent work on the PDR zinc clusters has focused on identifying which individual proteins are responsible for responding to different small molecules by swapping the native DNA binding domain for Gal4s [65]. This approach decouples native DNA binding and heterodimerization from the protein's function, allowing for closer examination of *in vivo* protein function without PDR network interactions. These experiments established Pdr1 as the most promiscuous activator in response to different small molecules as anticipated, while Yrr1 and Stb5 appear to be specific for either the mycotoxin ochratoxin A, or hydrogen peroxide, respectively. Future work utilizing similar approaches to this on all of the PDR zinc cluster proteins would solidify what stimuli each TF is responsible for.

Yeast adaptation to oxidative stress and metal stress are intimately intertwined

Keeping peroxides and other reactive oxygen species (ROS) at very low intracellular concentrations mitigates damage to the cell's DNA, proteins and lipids. ROS are generated endogenously by the mitochondria or from exposure to exogenous stresses such as oxidant molecules or metals [66,67]. Metal toxicity in yeast is one of the primary sources of oxidative stress, either through the generation of reactive oxygen species or unbalancing of the cell's antioxidant pool [68–70]. The vacuolar ABC transporters Ycf1 and Vmr1 play a significant role in alleviating metal toxicity by sequestration of metal-glutathione conjugates to vacuoles [71,72]. In *S. cerevisiae,* Ycf1, Vmr1, and other ABC transporters are regulated by a set of TFs that sense cellular levels of ROS and toxic metals (Fig. 1).

Yeast activator protein (Yap) TF Yap1 plays a key role as a regulator of the yeast oxidative stress response, turning on antioxidant genes [73]. In non-stress conditions, Yap1 is shuttled between the nucleus and cytoplasm by the exportin Crm1/Xpo1 and the importin Pse1 [74,75]. During oxidative stress, Hyr1/Orp1 forms an intermolecular disulfide bond to Yap1, which is then converted to an intramolecular disulfide bridge between Yap1 Cys598 and Cys303 within the within the N terminus cysteine-rich domain (nCRD) [76,77]. Additionally, a disulfide bond is formed between Cys629 and Cys310, linking the nCRD with the C terminus CRD (cCRD) (Fig. 2). These newly formed bonds masks the Yap1 nuclear export signal, resulting in rapid accumulation in the nucleus [78]. One of the primary gene targets of Yap1 is *YCF1*, one of the vacuolar ABC transporters responsible for transport of metal-glutathione conjugates [79]. Just like *YCF1*, the two PDR ABC transporters, *SNQ2* and *PDR5,* both possess Yap1 response elements in their promoters [80].

Yap2/Cad1 is an ohnolog of Yap1, with similar functions in oxidative and metal stress [81]. Yap2 has overlapping, but distinct gene targets from Yap1 during oxidative stress [82]. While Yap2 activation by hydrogen peroxide remains unclear, activation of Yap2 by metal toxicity occurs through a direct interaction of Yap2 with metals. Like Yap1, Yap2 is primarily localized in the cytoplasm in unstressed cells, but rapidly accumulates in the nucleus once its nuclear export signal is masked. This masking event is not dependent on disulfide bond formation from oxidative stress, but rather direct binding of cadmium to Cys391, Cys356, and Cys387 in the cCRD (Fig. 2) [83]. Yap1 and Yap2 regulate the same ABC transporters, *YCF1, SNQ2,* and *PDR5* with similar effects on expression. Both SNQ2 and *PDR5* are rapidly expressed in response to heat shock stress, dependent on the presence of both Yap1 and Yap2. Deletion of both Yap TFs delays their expression during early (< 1 h) heat shock stress

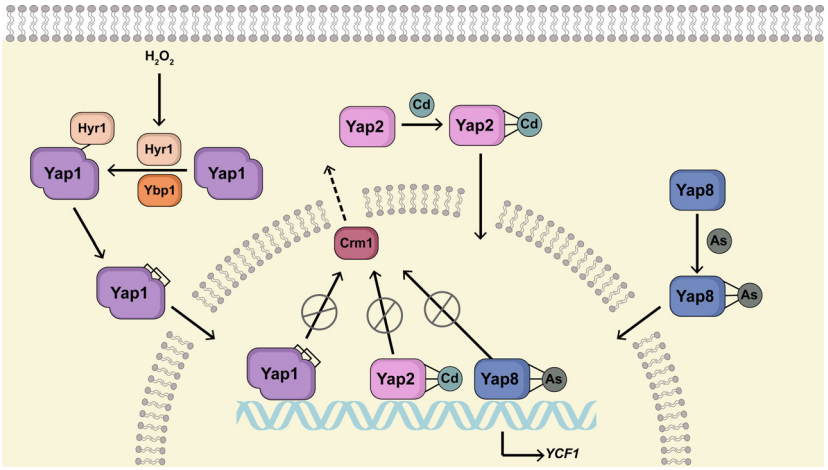

Fig. 2. Yap1, Yap2, and Yap8 regulate the yeast oxidative and metal stress response regulon by redox dependent nuclear export. In the presence of H2O2, a cysteine of Hyr1 is oxidized to sulfenic acid, which generates an intermolecular disulfide bond between Hyr1 and Yap1 facilitated by Ybp1. This disulfide bond is converted to form two intramolecular disulfide bonds linking the Yap1 N terminus cysteine-rich domain (nCRD) with the C terminus CRD. These newly formed bonds mask the Yap1 nuclear export signal preventing export by Crm1, leading to rapid accumulation in the nucleus. Yap2 and Yap8 have a similar mechanism of nuclear export signal masking by direct binding of either cadmium or arsenic, respectively, to three conserved cysteines, preventing nuclear export by Crm1.

[80]. Null mutants of either Yap1 or Yap2 behave the same as wild-type, suggesting that they are redundant regulators of heat shock induced *SNQ2* and *PDR5* expression. Investigation of other Yap1 regulated genes found that their heat shock induced expression is also dependent on Yap1 [84]. Increased temperatures enhance oxygen respiration in mitochondria leading to an increase in intracellular oxidation level that is sensed by both Yap TFs [84].

Yap8/Arr1 is another member of the Yap family of TFs that is essential for metal detoxification. Unlike Yap1 and Yap2, Yap8 does not play a role in oxidative stress, and instead is the yeast master regulator of arsenic detoxification [70,85]. Yap8 possesses 3 conserved cysteines Cys132, Cys137, and Cys274 that are essential for induction of Yap8 targets, presumably through direct binding of arsenic (Fig. 2) [85]. There have been conflicting reports of whether arsenic causes accumulation of Yap8 in the nucleus similar to Yap2, or if it is constitutively nuclear located [70,85]. As one of the primary methods of metal detoxification, *YCF1* is also regulated by Yap8 [86]. While Yap8 is responsible for only arsenate detoxification, Yap1 and Yap2 have broader abilities with the capability of sensing both the presence of metals and oxidant molecules by the status of their cysteines. Together, Yap1, Yap2, and Yap8 induce the expression of *YCF1*, *PDR5*, and *SNQ2* in response to a variety of toxic metals.

Msn2 and Msn4 encode homologous (32% identity) TFs with overlapping roles in regulating general stress response in *S. cerevisiae* by binding to stress response elements (STREs) [87–89]. Both TFs appear to be master regulators of the general stress response, modulating the expression of nearly 200 genes in response to different environmental stress such as heat shock, oxidative, and osmotic stress [90,91]. In response to stress, Msn2/4 are hyperphosphorylated and relocalize to the nucleus with periodic nucleocytoplasmic shuttling behavior [92–94]. Msn2/4 nuclear import is suppressed by phosphorylation of the nuclear localization sequence (NLS) by the cAMP-PKA pathway in basal conditions until nutrient starvation reverses NLS phosphorylation [95]. Despite sharing the consensus STRE, Msn2/4 targets are distinct for different stress conditions, suggesting that specific phosphorylation states or other interaction partners may control which STRE regulated genes are induced [90].

The vacuolar ABC transporter *VMR1,* which contributes to multidrug resistance and control of metal stress possesses two STREs in its promoter [72]. Deletion mutants of *MSN2/MSN4* lose the expected stimulated expression of *VMR1* by cadmium and zinc, suggesting that Msn2/4 are indeed regulators of *VMR1*. Msn2 also decorates the two STREs in the promoter of *PDR15* where it induces *PDR15* expression in response to heat shock, low pH, and high osmolarity [96]. Additional microarray and chromatin immunoprecipitation followed by microarray (ChIP-chip) or high-throughput sequencing (ChIP-seq) experiments on Msn2/4 indicate possible regulation of expression of *PDR5, PDR10, PDR11, and STE6* [72,95,97–107]. These ABC transporters may be part of the different subnetworks of genes induced by Msn2/4 in response to specific stresses. Msn2/4 represents a Yap-independent pathway for sensing oxidative stress generated by hydrogen peroxide and metals.

While *STB5* was first identified for its role in drug resistance by regulation of *PDR5* and SNQ2, it appears that this zinc cluster protein's primary function is similar to *YAP1*. ChIP-chip experiments revealed that Stb5 only shares a few genes with Pdr1 and Pdr3, with Stb5 binding to 100 genes not recognized by either Pdr1 or Pdr3 [108]. Interestingly, a large number of the Stb5 targets are proteins in the pentose phosphate pathway or involved with NADPH production. This suggests that Stb5 has a role in oxidative stress response as NADPH is used to regenerate glutathione, part of the cell's antioxidant pool [109]. *Δstb5* strains display sensitivity to oxidative stress induced by diamide or hydrogen peroxide [65,108]. Also, transcriptional activation from PDRE sites by hydrogen peroxide is abolished in *Δstb5* strains [65]. Stb5 activation is not dependent on any known kinase pathways, but rather on the integrity of the pentose phosphate pathway [108]. Like other zinc cluster proteins, Stb5 appears to be controlled by ligand binding, which may be intermediates in the pentose phosphate pathway whose concentrations change during oxidative stress. Unlike the Yap family and Msn2/4, Stb5 does not regulate either *YCF1* or *VMR1*, suggesting its role in oxidative stress response does not involve metal detoxification. Stb5 is the third pathway in *S. cerevisiae* for sensing disruptions in the cell's redox state, but unlike the Yap family that directly senses metals, Stb5 instead monitors the status of the pentose phosphate pathway that is responsible for NADPH production.

Together, these three different regulatory pathways are responsible for the expression of *YCF1*, *VMR1*, *PDR5*, and *SNQ2* in response to a variety of different forms of oxidative stress. *S. cerevisiae* possessing multiple distinct regulatory pathways to induce expression of these ABC transporters highlights their importance to the cell's response to oxidative stress. Pdr5 and Snq2 have been primarily investigated for their roles in drug resistance, but their regulation by both Yap1/2 and Stb5 strongly suggests they have a role in

oxidative stress response. It has long been speculated that the drug efflux pumps could have native substrates in addition to the drugs they transport [110,111]. Their role in oxidative stress response may be transport of native substrates, such as metabolites that are commonly modified by hydrogen peroxide and other ROS.

The involvement of ABC transporters in oxidative stress for other fungal organisms has not been extensively investigated, but recent work on the closely related *C. glabrata* has revealed changes in ABC transporter expression during oxidative stress. As a pathogen, *C. glabrata* frequently faces challenges from the host environment, including release of ROS from macrophages [112]. Like in *S. cerevisiae*, the *C. glabrata* Yap1 ortholog is one of the primary transcriptional regulators of genes required for oxidative stress adaptation [113]. Oxidative stress induces the expression of *CgYAP1*, *CgMSN2,* and *CgMSN4* along with either upregulation or downregulation of a number of ABC transporters [114]. While the role of the individual TFs has not been investigated yet, a ChIP-seq dataset on the *C. glabrata* Yap TFs identified *Cg*Yap1 binding to the *CgSNQ2* promoter, and *Cg*Yap2 binding to the *CgYCF1* promoter [115]. *Cg*Stb5 was found to play a similar role to *Sc*Stb5 in hydrogen peroxide resistance, but appears to be a negative regulator of ABC transporters [116]. Like in *S. cerevisiae*, the impact on oxidative stress adaptation by several ABC transporters is unclear in *C. glabrata*, but the same oxidative stress TFs appear to regulate ABC transporter expression in both species.

S. cerevisiae adapts to lipophilic weak acid stress *via* the efflux pump Pdr12

Weak organic acids are naturally occurring compounds that have long been used to prevent microbial growth. At low pH, weak acids can enter the cell by passive diffusion in their undissociated RCOOH state. Once inside the cell, the higher pH dissociates the weak acids resulting in release of protons and cytoplasmic acidification. Accumulation of intracellular weak acid anions and low cytoplasmic pH can inhibit glycolysis through inactivation of phosphofructokinase, resulting in a fall in cellular ATP concentrations leading to reduced cellular functions or cell death [117–119]. While many weak organic acids are harmful to yeast, lipophilic weak acids that enter the cell by passive diffusion across the cell membrane are the most potent inhibitors of cell growth [120].

Saccharomyces cerevisiae can adapt to stress caused by exposure to lipophilic weak acids such as sorbate or benzoate through induction of expression of the heat shock protein Hsp30 and the ABC transporter Pdr12 [8,121]. Despite its homology with Pdr5 and Snq2, Pdr12 has distinct nondrug substrates. Pdr5 and Snq2 have hundreds of different hydrophobic drug substrates, while the Pdr12 substrate spectrum appears limited to water-soluble monocarboxylic acids. In unstressed cells, Pdr12 is present at very low levels, but becomes one of the most abundant membrane proteins in response to weak acid stress [8,122]. The rapid induction of *PDR12* following lipophilic weak acid stress is primarily mediated by a zinc cluster TF, War1 [122,123]. Like the transporter it regulates, War1 function appears to be limited to responding to weak acids. War1 is constitutively located in the nucleus, where it becomes phosphorylated and increases promoter binding during lipophilic weak acid stress [122,123]. It is not yet clear whether hyper-phosphorylation is the cause or result of War1 activation, but nevertheless is tightly linked to the activation of War1 [122]. In the presence of a weak acid *in vivo*, a CFP-War1-YFP construct undergoes a conformational change that appears linked to increased promoter binding [123]. *PDR12* is also regulated by the TF Haa1, which is involved in the yeast stress response to hydrophilic weak acids such as acetic acid and lactic acid [124,125]. Haa1 target genes also include *TPO2* and *TPO3*, members of the major facilitator superfamily of transporters, that appear involved in the export of acid anions [126]. Recent work by Kim *et al.* revealed that *PDR12* is induced by acetic acid in a Haa1-dependent manner, while induction by other weak acids is primarily dependent on War1, with minor contributions from Haa1 [127]. Both Haa1 and War1 bind weak acids in a manner reminiscent of their induction of *PDR12*, with Haa1 binding acetic acid stronger than sorbic acid and benzoic acid, while War1's binding is reversed. Like *S. cerevisiae*, the distally related spoilage mold *Aspergillus niger* also displays resistance to lipophilic weak acids. The zinc cluster TF WarA and the ABC transporter PdrA were identified in *A. niger* that function in an analogous manner to War1 and Pdr12 in *S. cerevisiae* despite low sequence identity [128]. Complementation of a *S. cerevisiae Δpdr12* strain with *pdrA* recovered resistance to weak acids in a similar manner to *PDR12* [128]. While Pdr12 mediated weak acid export is necessary for survival during lipophilic weak acid stress, complete adaptation requires plasma membrane alterations to limit diffusional uptake of the weak acids.

Aus1 and Pdr11 import sterols in anaerobic growing conditions

Sterols are a major class of membrane lipids that are essential for proper structure and function of plasma

membranes [129]. In *S. cerevisiae,* the predominant sterol ergosterol can be synthesized by yeast cells in aerobic conditions. However, in the absence of oxygen ergosterol cannot be synthesized, so yeast must acquire sterols from the environment to survive [130]. Lewis *et al*. identified a mutation in the C terminus of TF Upc2 with 10- to 20-fold increase in sterol uptake when compared to WT [131]. Subsequently, an ohnolog (45% identity, 57% similarity) Ecm22 was identified with similar roles in sterol uptake [132]. Upc2-dependent sterol uptake was found to rely on the ABC transporter ohnologs *AUS1* and *PDR11* [133]. Both Upc2 and Ecm22 bind the 7bp sterol response element (SRE) present in the promoters of *AUS1* and *PDR11*, as well as other genes involved in sterol metabolism [133,134]. Two SREs are present in the *UPC2* promoter, leading to increased expression when cellular sterol concentrations are low [135]. The crystal structure of the Upc2 C-terminal domain has provided key insights into its regulatory mechanism [136]. This domain is a novel 11 helical bundle that contains a binding pocket for ergosterol or dehydroergosterol. This lipid-binding domain also plays a role in Upc2 dimerization through formation of a hydrophobic dimerization interface. The lipid-binding domain of Upc2 appears to mask the NLS when lipid is bound, resulting in cytoplasmic localization until sterol levels are depleted (Fig. 3) [136]. The previously discovered C-terminal G888D mutation disrupts sterol binding and is constitutively nuclear localized, supporting the theory that Upc2 is regulated by compartment localization. Like Upc2, Ecm22 also possesses a lipid-binding domain and translocates to the nucleus in response to sterol depletion [136].

Several additional TFs are either regulators of sterol transport or are responsible for repressing transport in aerobic conditions. In the presence of oxygen, heme is synthesized and bound by the TF Hap1 that induces expression of aerobic genes including *ROX1* (Fig. 3A) [137]. Rox1 represses the expression of numerous anaerobic genes including *UPC2* and *SUT1* [137,138]. Sut1 is another zinc cluster TF that regulates many of the same anaerobic genes as Upc2, including *AUS1* [139]. In aerobic conditions, transcription of *AUS1* and *PDR11* is also directly repressed by Mot3 [140]. A drop in oxygen concentration decreases heme levels, which transforms Hap1 into a repressor of *ROX1* and *MOT3* expression (Fig. 3B) [141,142]. The decrease in Rox1 and Mot3 levels increases the expression of *UPC2*, *SUT1,* and the sterol importers *AUS1* and *PDR11*. Together, these transcriptional activators and repressors coordinate the expression of either the ergosterol biosynthetic pathway or expression of ergosterol importers, depending on the availability of both

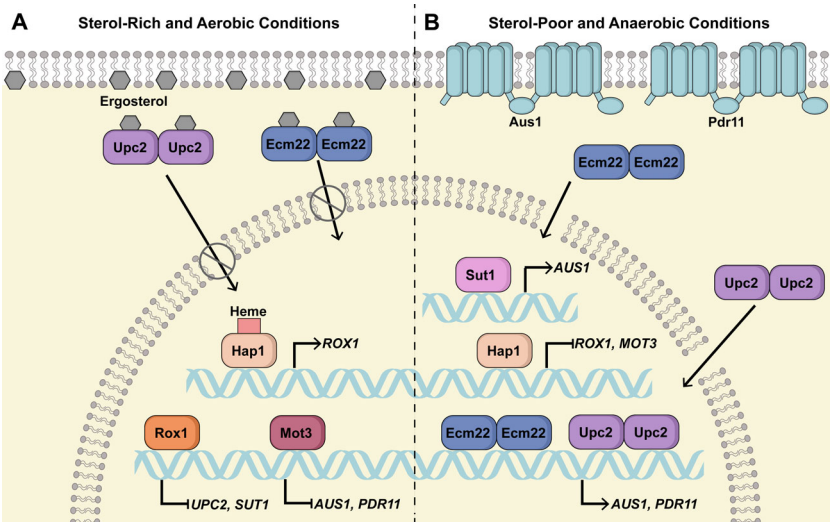

Fig. 3. Transcription of the sterol importers AUS1 and PDR11 is dependent on depletion of cellular oxygen and sterol levels. (A) In aerobic conditions, S. cerevisiae is capable of synthesizing sterols that are bound by Upc2 and Ecm22, keeping them localized in the cytoplasm. Hap1 is bound to heme in oxygen-rich conditions, increasing the expression of ROX1, which represses transcription of UPC2 and SUT1. Expression of AUS1 and PDR11 is also repressed by Mot3. (B) When cellular oxygen levels are low, sterol synthesis is disrupted leading to depletion of ergosterol, which results in Upc2 and Ecm22 nuclear import. Accumulation of nuclear Upc2 and Ecm22 induces transcription of AUS1 and PDR11. The reduced concentration of heme in anaerobic conditions switches Hap1 to a repressor of ROX1 and MOT3. AUS1 expression is also induced by Sut1 that is expressed in the absence of Mot3.

oxygen and ergosterol. A comprehensive summary of regulators of ergosterol synthesis and import are beyond the scope of this review, but have recently been reviewed elsewhere [143,144].

Sterol synthesis and import are essential for survival of many fungal organisms beyond *S. cerevisiae*. Upc2 homologs (*UPC2A* and *UPC2B*) have been of particular interest in *C. glabrata*. In addition to regulating sterol import, they regulate many proteins in the ergosterol synthesis pathway that are inhibited by a common class of antifungal drugs, azoles [145]. The Upc2A/B and *Cg*Aus1 behave in a similar manner to their homologs in *S. cerevisiae* where they are responsible for import of sterols in anaerobic conditions [145–147]. Additionally, *Cg*Aus1 is induced by fluconazole, and if exogenous cholesterol or ergosterol are present, wild-type *Cg*Aus1 strains are highly resistant to fluconazole treatment [147,148]. A notable difference between Upc2A and *Sc*Upc2 is the regulation of *CgPDR1* by Upc2A. It has been demonstrated recently that when sterol synthesis is blocked Upc2A binds to the *CgPDR1* promoter and induces transcription [149]. A fungal pathogen of cereal crops, *Fusarium graminearum* possesses several Upc2 orthologs, but its ergosterol biosynthesis gene network is instead regulated by a different zinc cluster TF FgSR [150]. FgSR orthologs and their DNA binding elements appear to be conserved across *Sordariomycetes* and *Leotiomycetes* fungi where they occupy similar roles to Upc2 in *Saccharomycotina* fungi.

PDR ABC transporters traffic lipids between the lipid monolayers of the plasma membrane

In addition to sterol import, other yeast ABC transporters play significant roles in plasma membrane homeostasis. Maintaining the appropriate lipid composition is required for membrane protein activity, and alteration of the composition increases yeast viability during heat shock, osmotic shock, and ethanol stress [151–154]. Many types of lipid molecules are not equally distributed between the inner and outer leaflets of the lipid bilayer but rather are localized asymmetrically. This asymmetric organization contributes to several cellular processes including membrane budding, endocytosis, and cellular signaling [155,156]. Both membrane proteins and lipids are also asymmetrically distributed laterally across the cellular membrane, where they are frequently localized into different membrane microdomains with specific functions [157–160]. ABC transporters along with P_4-ATPases are the primary classes of membrane proteins that are responsible for eukaryotic asymmetric transport of phospholipids [161–163].

Pdr5 and Yor1 were the first yeast ABC transporters identified to be involved in the movement of lipids from the inner to outer membrane (flop) of phosphatidylethanolamine [164,165]. More recently, the ABC transporter Pdr18 was found to control the sterol composition of the plasma membrane and hence the membrane permeability [166,167]. Deletion of *PDR18* increases plasma membrane permeability, resulting in sensitivity to herbicides and weak acid stress [166,167]. Aus1 and Pdr11 physically interact with the major yeast sterol *O*-acyltransferase Are2 in a membrane microdomain [168]. This interaction increases sterol esterification by Are2 that is used to store excess sterols into cytoplasmic lipid droplets for storage. Other PDR ABC transporters have less clear roles in membrane homeostasis, such as Pdr10 which appears to influence the localization or activity of different membrane proteins, including Pdr12 [169].

Like the ABC transporters that they regulate, a number of TFs have been implicated in lipid bilayer homeostasis. Detergents and other compounds that damage or perturb the plasma membrane increase Pdr1- and Pdr3-dependent transcription of PDR genes, including Pdr5 and Pdr15, which are required for resistance to nonionic detergents (Fig. 1) [170]. Examination of the cytoplasmic proteins regulated by Pdr1 and Pdr3 lends additional evidence that the PDR network is involved in membrane homeostasis. *PDR16* is a phosphatidylinositol (PI) transfer protein that shuttles PI between different membrane compartments and is involved in resistance to different azole drugs [171,172]. Induced expression of *PDR18* in the presence of herbicides is dependent on Pdr3 and Yap1 [166]. While Yap1 has no other known role in lipid bilayer homeostasis, Pdr18 reduces sensitivity to several divalent cations including cadmium, likely due to changes in membrane permeability. The accessory zinc cluster protein Rdr1 was originally identified as a repressor of drug resistance, but its function may be a regulator of membrane homeostasis. In addition to *PDR5* and *PDR15,* Rdr1 regulates only three other genes: *PHO84*, a MFS phosphate/H symporter, *RSB1*, a glycerophospholipid efflux transporter, and *PDR16*. Given the function of these three genes, along with Pdr5 and Pdr15's roles in PE transfer and detergent resistance, it is likely that Rdr1 is a regulator of plasma membrane maintenance [164,173]. The roles of ABC transporters in yeast environmental adaptation include not only export of drugs, metals, and weak acids, but also alterations of the lipid bilayer composition.

ABC transporters are required for cellular signaling

S. cerevisiae communicate with each other through two cell-to-cell processes: the mating response pathway and quorum sensing. *S.* cerevisiae exists as either diploid or one of two haploid mating types, *MAT***a** or *MAT*α. *MAT***a** and *MAT*α cells each produce a peptide mating factor that is secreted into the extracellular environment that is capable of being sensed by the other haploid mating type. When exposed to the opposite mating factor, the haploid cells arrest their cell cycle and grow a projection toward the source of the mating factor until they make contact with the other haploid cell. On the other hand, quorum sensing coordinates the behavior of *S. cerevisiae* in response to the density of the population. Yeast cells secrete signaling molecules into the extracellular environment which eventually reach a critical concentration once the population reaches a certain density. This results in a switch in behavior across the entire population, such as transitioning to filamentous growth to forage for nutrients in response to nitrogen starvation [174].

One of the most unique yeast ABC transporters is Ste6, which is responsible for the secretion of peptide a-factor from *MAT***a** cells [7,175]. Expression of Ste6 is regulated by two mating pathway TFs, Ste12, and Matα2. In the presence of exogenous mating pheromone the yeast MAPK cascade is triggered, culminating in the activation of Ste12 by phosphorylation of Ste12 and its binding partners Dig1 and Dig2 [176,177]. Ste12 induces transcription of mating pathway proteins by binding to pheromone response elements [178]. Despite the presence of Ste12 in both *MAT***a** and *MAT*α, expression of Ste6 in *MAT*α is repressed at least 150-fold [179]. *MAT*α cells express Matα2, a transcriptional repressor for *MAT***a** specific mating genes, which includes *STE6* [179]. Together, Ste12 and Matα2 control the expression of Ste6 so that it is present in *MAT***a** cells but not *MAT*α or diploid **a**/α.

In addition to its role in drug resistance, the PDR network also appears to be connected with a specific type of quorum sensing involving growth arrest when switching from glucose to nonfermentable carbon sources. This growth arrest, known as the diauxic shift, is delayed in *Δpdr5Δsnq2* cells implicating their role in this behavior. Resuspending *Δpdr5Δsnq2* cells in conditioned media from a WT culture results in growth arrest similar to WT, demonstrating that Pdr5 and Snq2 are likely responsible for export of molecules that signal the diauxic shift [180]. The role of Pdr5 and Snq2 in the diauxic shift appears to be regulated by Pdr1 and its interaction partners Ssz1 and Zuo1. During the discovery of the PDR network, a Hsp70 chaperone Pdr13/Ssz1 and its Hsp40 partner Zuo1 were identified that increased multidrug resistance through activation of Pdr1 [181,182]. This Hsp70/40 pair is normally associated with the ribosome where they participate in co-translational folding. However, once disassociated from the ribosome, they activate Pdr1 in a nonchaperone manner by directly binding to it [180,182]. A yeast two-hybrid assay demonstrated this was a direct interaction of Pdr1 with Ssz1 and Zuo1 [180]. In the case of Zuo1, the partial unfolding of a C-terminal helical bundle results in both its dissociation with the ribosome and binding to Pdr1 [183]. This Zuo1/Ssz1 mechanism for activating Pdr1 mediated transcription appears to be a way to couple translational stress to an earlier growth arrest that may increase cell survival.

ABC transporters and their transcriptional regulators also appear to play a role in quorum sensing in other fungal organisms. One of the first eukaryotic quorum sensing molecules identified was farnesol, produced by the fungal pathogen *Candida albicans* [184]. The synthesis of farnesol and its effects on *C. albicans* virulence-related traits including biofilm formation have been extensively characterized and reviewed elsewhere [185]. Recently there has been several publications linking *C. albicans* drug efflux ABC transporters to farnesol concentrations. Expression of the ABC transporters *CDR1* and *CDR2* is induced in the presence of farnesol, a process that is dependent on their transcriptional regulators Tac1 and Znc1 [186,187]. While the role of Cdr1 and Cdr2 in the farnesol response remains unclear, there is some initial evidence that Cdr1 may be one of the farnesol transporters [188]. Interestingly, the bacterium *Pseudomonas aeruginosa* that can co-exist at *C. albicans* infection sites releases a quorum sensing molecule that also increases the expression of *CDR1* and *CDR2* in the presence of the antifungal fluconazole [189]. Fungal quorum sensing has been primarily studied in *S. cerevisiae* and *C. albicans,* but further study of quorum sensing in other fungi may reveal additional ABC transporters involved in cellular signaling.

Prospective ABC regulators

In addition to the established transcriptional regulators of *S. cerevisiae* ABC transporters, there are a number of candidate TFs, the roles of which in ABC transporter expression have not been studied. A search of the YEASTRACT database for TFs with preliminary evidence of DNA-binding and expression identifies a

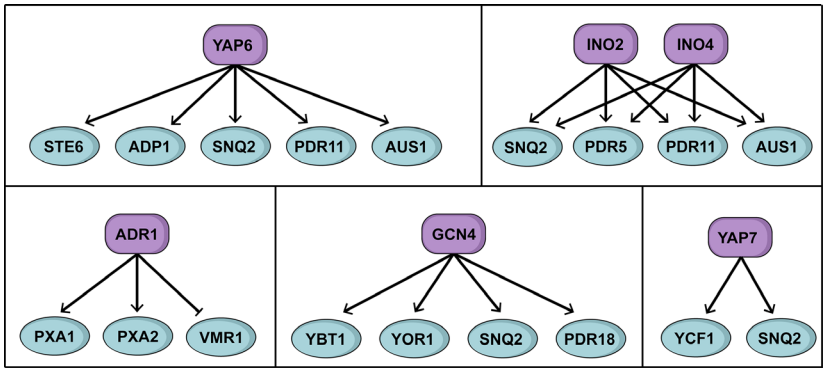

Fig. 4. Proposed new transcriptional regulators of *S. cerevisiae* ABC transporters. Six *S. cerevisiae* transcription factors (purple) appear to each regulate several ABC transporters (blue), despite limited study of these interactions. The six transcription factors were identified by searching the YEASTRACT database for regulators of any *S. cerevisiae* ABC transporters. Proteins were included based on direct binding evidence observed in multiple publications, and involvement in the same cellular processes as the transporters they may regulate.

number of TFs that may regulate ABC transporters [190]. There are 6 of particular interest, Adr1, Gcn4, Ino2/4, Yap6, and Yap7 based on known function, and evidence that they regulate multiple ABC transporters (Fig. 4).

The carbon source-responsive TF Adr1 regulates the expression of genes required for ethanol, glycerol, and fatty acid utilization along with peroxisomal proteins [191–193]. ChIP-chip experiments have identified Adr1 binding to the promoters of *PXA1* and *PXA2*, the two yeast peroxisomal ABC transporters responsible for peroxisome fatty acid import [194–197]. Microarray data identify Adr1 as a positive regulator of *PXA1* and *PXA2*, which aligns with its established role as a peroxisome regulator. Additionally, Adr1 appears responsible for the repression of the expression of *VMR1* when glucose is a carbon source [72]. The vacuolar ABC transporters *YCF1* and *VMR1* have similar cellular functions, but each function predominantly on either glucose or a respiratory substrate (ethanol or glycerol), respectively [72].

Gcn4 is a member of the bZIP family that activates the expression of amino acid biosynthetic pathway genes in response to amino acid starvation [198]. Gcn4 activity is not confined to biosynthetic pathways, as it targets other genes related to amino acid biosynthesis including peroxisomal proteins, amino acid transporters, and mitochondrial carrier proteins [199]. ChIP-chip and microarray data implicate Gcn4 as a regulator of *PDR18, SNQ2, YOR1,* and *YBT1* [200–202]. Also, induction of *PXA2* in response to histidine starvation is dependent on Gcn4, though it may not be a direct interaction [199]. While *PDR18, SNQ2, YOR1,* and *YBT1* are not involved in amino acid biosynthesis, they may be regulated by Gcn4 in order to eliminate any inhibitors of amino acid biosynthesis.

Ino2 and Ino4 are basic helix-loop-helix TFs that form a heterodimeric complex to regulate phospholipid biosynthetic genes in response to inositol depletion [203,204]. Microarray expression and ChIP-seq datasets include *AUS1, PDR11, PDR5,* and *SNQ2* as genes likely to be regulated by Ino2/Ino4 [205,206]. *AUS1, PDR11,* and *PDR5* each have roles in controlling plasma membrane composition as discussed previously with their regulators *RDR1*, *UPC2*, and *ECM22*.

Two of the other member of the Yap family, Yap6 and Yap7, are present at the promoters of several ABC transporters in ChIP experiments. While much remains to be discovered about Yap6 and Yap7, it is established that they play a role in hyperosmotic and nitrosative (reactive nitrogen species) stress [207–210]. Yap7 binds to the promoters of *YCF1* and *SNQ2*, similar to other Yap TFs, while Yap6 has been found at the promoters of different ABC transporters such as *SNQ2, STE6, ADP1, AUS1,* and *PDR11* [196,211,212]. Further work on the function of these two Yap TFs should clarify whether they indeed are regulators of yeast ABC transporters.

Concluding perspectives

Despite intensive research on yeast ABC transporters over the past four decades, there remains aspects of their transcriptional regulation left unexplored. Many ABC transporters lack validated transcriptional regulators, including nearly half of all *S. cerevisiae* ABC transporters (Fig. 1). However, information on new potential regulators may be discovered through the ChIP-seq datasets available for *S. cerevisiae* (Fig. 4).

When we look at closely related fungal species, we find that ABC transporter regulation appears conserved. For example, within the *Saccharomycetaceae* family PDREs are present in the promoters of PDR transporter homologs, suggesting that the PDR TFs have conserved functions [213,214]. Even in more distantly related and underexplored fungal species, there is clear evidence of transcriptional regulation of ABC transporters despite the lack of TF homologs. Fungi outside of *Saccharomycetaceae* possess zinc cluster TFs with divergent amino acid sequences, such as Tac1 in *C. albicans* and atrR in *A. fumigatus*, yet fulfill similar roles in drug resistance. The lack of conservation of many ABC transporter regulators in fungi highlights the need to study them directly in the species of interest, as there are likely key differences in both mechanism and function.

Several PDR regulators are capable of forming heterodimers, but the role of heterodimerization has yet to be explored. Two prominent ABC transporters, Pdr5 and Snq2 possess many regulators and partake in many roles in the cell. In contrast, Pdr12 with its singular role in weak acid export possesses two transcriptional regulators. Due to the number of possible zinc cluster homodimers and heterodimers regulating each ABC transporter, in addition to Yap1 and Yap2, transcriptional regulation of Pdr5 and Snq2 has an increased level of complexity. The Pdr1/Pdr3, Pdr1/Stb5, and Pdr1/Rdr1 heterodimers may result in different regulation when compared to homodimers. ChIP-seq experiments isolating the different dimers, or single molecule experiments on mixed populations of TFs may identify differences in DNA binding between the possible dimers. The formation of heterodimers has been underexplored in other fungal organisms, but it may be of interest for ABC transporters regulated by several zinc cluster TFs.

In recent years, there has been increasing evidence that PDR ABC transporters control plasma membrane composition, but the roles of their transcriptional regulators in this process remain unclear. PDR TFs regulate a number of other proteins involved in lipid synthesis and transport, suggesting PDR ABC transporters are part of a broader network responsible for the modification of the plasma membrane in response to different environmental stresses. The different signals PDR TFs recognize to induce expression of genes to alter the plasma membrane remains underexplored. Also, clarity on what contribution each TF makes in this process may lead to a better understanding of their function. For example, Rdr1 is a repressor of *PDR5* and *PDR15* expression, but it is unclear if this repression is altered in different environmental conditions. Modern fluorescence microscopy techniques and environment-sensitive dyes could further identify the impact of ABC transporters and their regulators in both lipid bilayer composition and mechanical properties.

While it has not been a focus of this review, the interactions between TFs and components of the transcriptional machinery have been of growing interest in the field. In yeast and other eukaryotes, Mediator is a conserved multisubunit protein complex that is responsible for transducing signals from transcription activators to RNA polymerase II (while a detailed description of Mediator is outside the scope of this review, there is an excellent recent review of its function) [215]. *Sc*Pdr1, *Cg*Pdr1, and Tac1 all induce transcription by recruitment of the Mediator complex through binding of their activation domains to different Mediator subunits [32,216]. Recently, a small molecule inhibitor (iKIX1) of the *Cg*Gal11A Mediator subunit binding site for *Cg*Pdr1 activation domain was able to restore azole sensitivity in *CgPDR1* gain-of-function mutant strains [217]. iKIX1 was also able to inhibit the same interaction between *S. cerevisiae* Pdr1 and Gal11, preventing ketoconazole induced expression. Interestingly, amino acids in the Pdr1 activation domain that bind Gal11 are conserved across both *S. cerevisiae* zinc cluster TFs and Pdr1 homologs in other fungal species [218]. A similar approach for the different zinc cluster TFs involved in drug resistance in different fungi could prove effective in treatment of multidrug-resistant strains.

While the zinc cluster TF family is fungal specific, there are notable parallels between transcriptional regulation of *S. cerevisiae* and human ABC transporters. A close human functional homolog for *ScPdr5* is P-glycoprotein (P-gp), a well-characterized drug efflux pump [219]. In humans, the metazoan nuclear receptors appear to fulfill the same roles as zinc cluster proteins in regulating ABC transporters, such as PXR, which bind to a number of drugs to activate transcription of drug efflux pumps including P-gp [220–225]. PXR is not alone in regulating the human drug efflux ABC transporters, as at least two other nuclear receptors (CAR and FXR) are also involved [226,227]. Work on the *S. cerevisiae* ABC transporters and the TFs that regulate them has laid the groundwork for understanding transcriptional regulation of ABC transporters in fungi and beyond.

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