Antifungal resistance

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Sterol homeostasis in yeast

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The fungal sterol receptor and transcription factor Upc2 activates the transcription of ergosterol biosynthesis genes in response to ergosterol depletion in yeast. A structural and biochemical study reveals an Hsp90-dependent translocation activation mechanism of Upc2, with implications for triazole antifungal resistance.

The triazole antifungals, such as fluconazole, act by competitively inhibiting sterol demethylase, a key enzyme of the fungal ergosterol biosynthesis pathway. This results in both a reduction in cellular ergosterol and an accumulation of alternate sterols believed to be 'toxic' to the cell and produce the fungistatic activity observed against *Candida* species[1]. The major transcriptional regulator of ergosterol biosynthesis in *Candida* and certain other yeasts is the zinc cluster transcription factor Upc2. In this issue of *Nature Chemical Biology*, Tan et al. reveal the structures of Upc2 in complex with ergosterol from two yeast species, providing insight into the ligand-binding and activation mechanism, and shedding light on its constitutively activated state due to mutations that confer resistance[2].

Candida species are collectively among the most common cause of fungal infections in humans, ranging from oral and vaginal infections to serious invasive disease. Fluconazole and other triazoles are among the antifungal agents most widely used to treat *Candida* infections. At present, only two other classes of antifungals (the polyenes and echinocandins) are available for the treatment of invasive fungal infections. Resistance to all three antifungal classes has emerged, leading the US Centers for Disease Control to classify drug-resistant *Candida* species as a "serious threat" in their latest Antibiotic Resistance Threats Report, with the emerging multi-drug-resistant yeast *Candida auris* categorized as an "urgent threat".

Deletion of the gene encoding Upc2 results in hypersensitivity to fluconazole and other triazoles and an inability to upregulate key ergosterol biosynthesis genes under conditions of ergosterol depletion[3]. Moreover, activating mutations in the gene encoding Upc2 are common among resistant isolates of *Candida albicans* and have been shown to contribute to fluconazole resistance by increasing the expression of ergosterol biosynthesis genes, including that encoding the triazole target, sterol demethylase[4,5]. Tam et al. have advanced our understanding of how ergosterol levels are sensed by Upc2 and how this transcription factor is activated in response to reduced cellular ergosterol levels[5]. Previous work from this group reported the apo form of the Upc2 ligand-binding domain from *Saccharomyces cerevisiae* and proposed the hypothesis that when ergosterol is abundant, Upc2 is

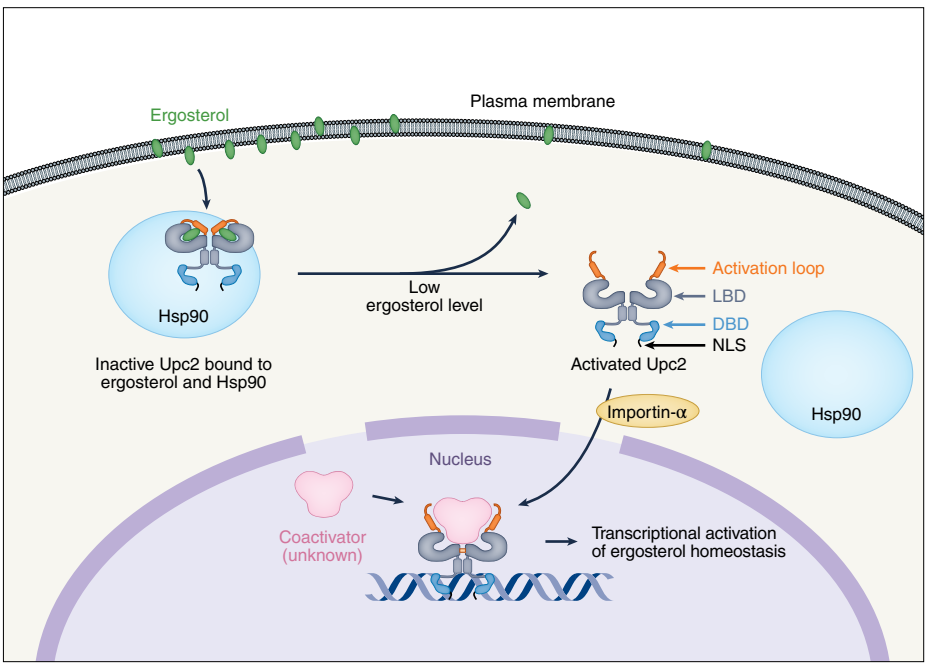

Fig. 1 | Schematic model of sterol sensing and transcriptional regulation by Upc2. When ergosterol is abundant, Upc2 binds to ergosterol and remains in the cytosol in an inactive form bound to Hsp90. Upon ergosterol depletion, Upc2 dissociates from Hsp90 in an activated form and translocates via importin-α to the nucleus, where it activates the transcription of genes required for ergosterol homeostasis. DBD, DNA-binding domain; LBD, ligand binding domain; NLS, nuclear localization signal. Figure adapted with permission from ref. [6], Springer Nature Ltd.

bound in an inactive state to ergosterol, whereas in ergosterol-limiting conditions, unbound Upc2 transitions to an active state, translocates to the nucleus and initiates transcription of its target genes[6]. In the present work, the authors solved the structures of ergosterol-bound, inactive Upc2 from *Candida glabrata* and *Lachancea thermotolerans*, showing that the conserved helix α12 and glycine-rich loop in the C-terminal tail are key to fungal sterol recognition. The helix α12 is flexible in the unbound form but is stabilized upon ligand binding. They further demonstrated a key role for the molecular chaperone Hsp90, showing that when bound to ergosterol, Upc2 associates with Hsp90, retaining its inactive cytosolic form. Dissociation of ergosterol from Upc2 releases Upc2 from Hsp90 through a conformational change, allowing Upc2 to translocate to the nucleus via importin α and activate the transcription of its target ergosterol biosynthesis genes (Fig. 1).

Mutations leading to the G888D amino acid substitution within the glycine-rich loop adjacent to helix α12 in *S. cerevisiae* Upc2 (ScUpc2) lead to upregulation of target genes and increased resistance to fluconazole, presumably through upregulation of the gene encoding the triazole target sterol demethylase[7]. Similar mutations have been found in fluconazole-resistant clinical isolates of *C. albicans*, where this represents an important mechanism of resistance. Tam et al. show here that that G898 in *C. glabrata*, which corresponds to G888 in ScUpc2, is critical for the binding of Upc2A (a Upc2 homolog in *C. glabrata*) to ergosterol, and that mutations in this region inhibit ligand binding through steric clashes[2]. This represents an important advance in our knowledge of how such mutations impart resistance, and may inform our understanding of how mutations in genes encoding other zinc cluster transcription factors that mediate resistance (such as Pdr1 in *C. glabrata* and Tac1 and Mrr1 in *C. albicans*) elicit their effects.

In addition to Upc2's role in fluconazole resistance, loss of Upc2 greatly enhances fluconazole susceptibility, both in susceptible and resistant isolates[3,8,9]. Activation of another zinc cluster transcription factor, Pdr1, drives fluconazole resistance in *C. glabrata* through its regulation of genes encoding drug transporters. Small-molecule inhibitors of its activation have been discovered that resensitize drug-resistant *C. glabrata* to triazole antifungals[10]. The work reported here by Tam et al., by providing a fuller understanding of how Upc2 is activated as ergosterol becomes limiting, points to similar opportunities for therapeutic intervention to hypersensitize *Candida* to the triazole antifungals and enhance, or in some cases reclaim, the utility of this important antifungal class[2].

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Competing interests

The author declares no competing interests.