

Review Article

Transcriptional Regulation of CDR1, a major efflux pump involved in multidrug resistance in *Candida albicans*

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ABSTRACT

Keywords

Multidrug resistance, MDR, CDR1, *Candida albicans*,

Drug resistant *Candida albicans* infection is an emerging problem in immuno-compromised individuals causes persistent Candidiasis. Over expression of *CDR1* is major mechanism of drug resistance in *Candida albicans*. Expression of *CDR1* is affected by various physiological and growth conditions. *CDR1* expression is tightly controlled by various transcription factors and through different mechanisms which acts in separate or in combination to regulate *CDR1* expression. This review summarizes the current knowledge about the transcriptional regulation of *CDR1* in *Candida albicans*.

Introduction

Candida albicans apart from being the most common human fungal pathogen is also the omnipresent component of the mammalian micro biome. In recent years, the incidence of fungal infection has shown a remarkable increase. *Candida albicans*, is one of the major fungus which is found to be responsible for skin infections (Morrison et al, 2006). *Candida albicans* exists as a commensal organism in healthy individuals but becomes pathogenic in immune compromised individuals. In United States, Candidiasis causes fourth most common nosocomial bloodstream infection.

Azoles are preferred compared to other drugs for treatment of *Candida* infections

due to its low toxicity, easy availability and increased efficacy. Azoles can be broadly classified in imidazoles and trizoles. Imidazole, generally used for skin infections, includes ketoconazole, miconazole and clotrimazole. The second class, trizoles includes fluconazole, posaconazole, voriconazole, itraconazole. Drugs from this class are used in the treatment of systemic infections. Azoles inhibit Erg11 (lanosterol alpha demethylase), inhibition of Erg11 leads to concomitant accumulation of methylated sterols and depletion of Ergosterol. Ergosterol is an important constituent of cell membrane that helps in maintaining membrane fluidity and the depletion of Ergosterol leads to inhibition of growth.

Failure in treatment of *Candida* infection has increased nowadays due to rise of azole drug resistance. Drug resistance is a condition where an organism is capable of surviving on one or more classes of drugs (Ghannoum et al, 1999). It is an emerging problem in *Candida* drug therapy (Morschhauser et al, 2010). Various mechanisms have been proposed for development of drug resistance for example, alteration in ergosterol biosynthetic pathway, over expression of efflux pumps, mitochondrial dysfunction etc. (Prasad et al, 2005). Over expression of efflux pumps is a prominent mechanism of drug resistance in *Candida albicans*. Major efflux pumps that are over expressed in drug resistance condition belongs to two super families ABC efflux proteins (CDR1, CDR2) and MFS efflux proteins (MDR1) (Calabrese et al, 2000),. CDR1 was the first ABC transporter to be identified in *C.albicans*. It has been shown that during drug resistance condition Cdr1 is over expressed (Prasad et al, 1995).

Regulation of CDR1 is an intricate process. Various cis and trans acting factors have been found to be regulating the CDR1 expression (Sanglard et al, 2009). To understand mechanisms of drug resistance it is important to understand the regulatory network governing MDR (Multi drug resistance) in *C.albicans*. Here we will discuss further the cis and trans acting factors involved in CDR1 regulation and mechanism through which CDR1 is regulated in *C.albicans*.

Role of CDR1 in *Candida albicans* Drug tolerance: Cdr1 (*Candida* Drug Resistance protein 1) is a close homologue of Human ABC transporter P- gp. It contains 1501 amino acids of molecular weight 169 Kd. CDR1 comprises two cytoplasmic nucleotide binding domain (NBD) and two transmembrane domains

(TMD) (Prasad R et al, 2012). It is a major extrusion pump in *C.albicans* (Prasad R et al, 2005). CDR1 exports azoles, derivatives of azoles are also able to efflux various structurally unrelated compounds. It transports phospholipids in an in-to-out direction. CDR1 helps cells in detoxification process by exporting out cellular metabolites. CDR1 exports beta-estradiol, and corticosteroid but is unable to export progesterone. CDR1 also plays role in ion transport. Expression of CDR1 was found higher in drug resistant isolates and transient exposure to azoles. Increased expression of CDR1 leads to outward efflux drugs from the cells. Over expression of CDR1 does not allow accumulation of the critical concentration of drugs which is necessary to kill cells.

Cis acting elements regulating CDR1 expression

Constitutive over expression of CDR1 is one of the prominent factors which lead to azole resistance. By promoter deletion analysis and in silico analysis various regulatory sequences were identified in CDR1 promoter. cis acting element regulates CDR1 in both positive and negative manner.

DRE (Drug Responsive Element): In ABC transporters genes, CDR1 and CDR2, upregulation is facilitated by a common drug responsive element (DRE) as determined by 5' deletions and Renilla luciferase reporter assays. A consensus of 21 bp with the sequence 5'-ACGGA(A/T)TATCGGATATTTTTTTT-3' having no equivalent to known eukaryotic regulatory sequence is shared by DRE (Micheli D et al, , 2010). In CDR1 promoter DRE positioned -357 to -417 upstream to TSS. DREs function as independent elements when inserted into a non-responsive promoter. The importance

of the DRE domain in CDR1 and CDR2 regulation was validated by loss of reporter activities when the different promoter deletion constructs were analyzed in the background of an azole-resistant strain. DRE domains involved in regulation of CDR1 genes were not oestradiol specific as antifungal such as amorolfine or terbinafine could also induce CDR1 genes (Micheli D et al, 2010). DRE domains identified are functionally active for different unrelated compounds which imply presence of a common regulatory pathway must be activated in *C.albicans* in response to the presence of different substances.

NRE (Negative Regulatory Element): A promoter can be negatively regulated by a repressor actively by binding to the activity site or passively by preventing an activator protein from binding to the specific sequence. Previous studies have identified a negative regulatory element (NRE), at position -272 to -265 upstream of the transcriptional start site of CDR1 that controls its basal expression.(Gaur NA et al, ,2004).Mutation and deletion analysis affirmed the sequence of NRE as (-ccaaCTGATTGAaact-). A 55 kDa nuclear protein (NREBP), which specifically interacts with NRE, was reported. Deletion or mutation of this sequence resulted in enhanced promoter activity, hence validating the role of NRE in CDR1 expression. However, this repression was overcome in azole-resistant clinical *C.albicans* isolates as binding of the NREBP to the CDR1 promoter is impaired leading to CDR1 expression (Gaur NA et al, 2005).

SRE (Steroid Responsive Element): CDR1 of *Candida albicans* is differentially regulated by various drugs and steroids (Krishnamurthy S et al,

1998). Promoter analysis manifested a Steroid Responsive Region, conferring β -oestradiol and progesterone inducibility on the CDR1 promoter which is located -696 to -521 bp upstream of the transcription start site.SRR comprises two distinct regions namely SRE1 and SRE2. SRE1 responds to progesterone while SRE2 responds to both progesterone and β -oestradiol but are not activated by drugs. (Karnani N et al, 2004). In silico comparison of the SRE1/2 with the promoter sequences of other MDR (CDR2 and PDR5) and non-MDR (HSP90) steroid-responsive genes revealed a similarity with respect to conservation of three 5 bp stretches (AAGAA, CCGAA and ATTGG).

BEE (Basal Expression Element): In CDR1 promoter, BEE is responsible for its basal expression. By promoter deletion analysis it was found that a domain -860 to -810 upstream from TSS containing an element (BEE) regulates basal promoter activity of CDR1 promoter (Puri N et al, 1999). This domain does not contain consensus sequences of DRE but activate CDR1 upon exposure of drugs and deletion of this domain results in drug unresponsiveness (Gaur NA et al, 2004).

MSE (Middle Sporulation Element): In *C.albicans* CDR1 promoter contain three potential MSEs (CRCAA) located 270, 438, and 835 bp upstream from the transcription initiation site. In *S. Cerevisiae* it was shown that Ndt80 directly regulate its target genes via the Mid Sporulation element (MSE) by binding through its DNA binding domain (Wang JS et al, , 2006). In silico analysis reveals that target promoters of CaNdt80 bound to the middle sporulation element, 5_-gNCRCAAAY-3_, similar to *S.cerevisiae*. (Here lowercase letter

denotes a semi conserve residue, N indicates any nucleotide, R indicates a purine and Y indicates either a thymine or a cytosine).

HSE (Heat Shock Element): in silico analysis reveals two potential heat shock element (HSE) in CDR1 promoter. HSEI placed -259 bp with consensus sequences TTCCCGAA and HSE II is present -128 bp away from Transcription Start Site with consensus sequences TTCTTGAA (Gaur NA et al, 2004). HSE also presents in CDR2, CDR3, PDR5 of *S.cerevisiae* and MDR1 of *Homo sapiens* all these genes have role in drug resistance.

Other Cis acting Element: in silico analysis of CDR1 promoter by using TESS programme revealed various putative transcription factor binding sites. AP-1, ERE, GRE, MDR1- NF- 1, pHRE, PhRE, PRE, RARE, TATA, YAP1 were the potential transcription factors binding site (Gaur NA et al, 2004). By using EMSA binding of some of the sequence has been confirmed but their role in CDR1 regulation is still unknown. By using promoter deletion analysis it was found that CDR1 promoter contains four upstream activating sequences domain and four upstream repressor domain (Gaur NA et al, 2004).

Trans acting Factors involved in CDR1 regulation: A trans acting element is a protein which regulates expression of one or more genes by binding to cis acting elements. trans acting element regulates expression through recognizing target sequence on promoter and by interacting with other transcription factors. In case of CDR1 regulation various transcription factors were identified which binds to CDR1 promoter and regulate its expression (as shown in figure 1).

Tac1 (Transcriptional Activator of CDR gene): Tac1 belongs to zinc cluster superfamily and it was the first trans acting factor identified as regulator of CDR1 expression. Tac1 is responsible for CDR1 overexpression during transient exposure to drug and in drug resistant strains. In *C.albicans* deletion of TAC1 gene abolish expression of CDR1 (Coste AT et al, 2004). Tac1 binds to CGG triplets which is presents in DRE element of CDR1 promoter through N terminal DNA binding domain. In *Candida* genome TAC1 is positioned upstream to mating type locus. GOF gain of function mutation in Tac1 leads to hyper activation of CDR1 expression (Coste AT et al, 2006). ChIP – chip experiment revealed Tac1 binds to 37 target promoters across *Candida* genome CDR1, CDR2, IFU5, PDR16, RTA3 are among them. Tac1 target promoter involved in lipid metabolism which is another important factor for drug resistance in *C.albicans* (Liu TT et al, 2007).

Ncb2 (Negative Co factor 2 beta subunit): Ncb2 is a beta subunit of NC2 complex (Negative co - factor 2). NC2 first identified as transcriptional repressor in human nuclear extract, is a heterodimeric complex comprising Bur6 and Ncb2. Ncb2 is conserved among eukaryotes and is essential for viability (Shukla S et al, , 2011). NC2 heterodimer acts as a molecular clamp, gripping the upper and lower surface of the TBP-DNA binding complex. In *C.albicans* it was shown that Ncb2 binds to CDR1 promoter in vivo. NCB2 conditional null mutants show enhanced azole resistances and increased CDR1 expression. This data indicates the positive role of NCB2 in CDR1 expression. Transcription level of NCB2 remains same in both azole susceptible (AS) and azole resistance (AR)

strains (Shukla S et al, 2011). Occupancy of Ncb2 is increased in azole resistant isolate at TATA box. This data suggests that Ncb2 mobilizes transcriptional complex upstream to TATA box on CDR1 promoter in drug susceptible strain. By using chip coupled with PCR experiment it was found that Ncb2 regulates similar set of target genes regulates by Tac1.

Ndt80 (Non di tyrosine 80): CaNdt80 mediates drug resistance by regulating the expression of CDR1 in *Candida albicans*. Lac Z reporter assay revealed that over expression of CaNDT80 increases the β -galactosidase activity of the CDR1 p-lacZ construct in *S. cerevisiae*. Further, overexpression of a fusion protein containing the potential trans-activation domain of CaNdt80 and the DNA binding domain of Ndt80 induced the expression of CDR1p-lacZ in *S. cerevisiae* (Wang JS et al, 2006). This TF bound also bound large number of gene promoters with various biological functions, such as cell wall, hyphal growth, carbohydrate metabolism, and the mitotic cell cycle as shown by genome-wide occupancy using chromatin immunoprecipitation coupled with high-density tiling arrays (Sellam A et al, , 2009) CaNdt80 can therefore be predicted to be a general transcription regulator having roles in activation as well as repression (Sellam A et al, 2010).

Upc2: Upc2 is major regulator of ergosterol biosynthesis process this transcription factor belongs to zinc cluster family. UPC2 is a close homologue of ECM22 of *S. cerevisiae*. Upc2 plays dual role in regulation acts as both activator and repressor regulates various cellular functions (Vasicek EM et al, 2014). Genome wide location analysis revealed that Upc2 binds to CDR1 promoter (Znaidi S et al, 2008). This interesting

finding suggests some link between ergosterol metabolic process and drug transporter activity.

Mechanisms for CDR1 activation / repression: In *C. albicans* CDR1 expression is highly controlled by various transcription factors and associated factors through various mechanisms. One or more than one mechanism contributes to CDR1 regulation. LOH (Loss of Heterozygosity), GOF (Gain of Function) mutation in Tac1, post transcriptional modification and other established and proposed mechanism are describe here.

Gain of Function Mutation and homozygosity: Gain of function (GOF) mutation in transcription factors regulation CDR1 expression is one of the mechanisms of CDR1 activation. Single point mutation (N977D) was the first gain of function mutation in TAC1 allele reported by (Coste AT et al, 2006). This mutation is present in C – terminal activation domain. This point mutation is sufficient to hyper activate CDR1. In another report, same group reported 17 different GOF mutations present at 13 different positions. Some of the mutations reported are G980E, N972D, A736V, T225A, N77D, G980W, A736T, N972S, N972I. Hyperactive allele possesses co dominance.

Homozygosity is the condition necessary for CDR1 overexpression (Coste AT et al, 2006). Possible reason for homozygosity is the duplication of hyperactive allele upon the exposure of drug later the loss of TAC1 wild type allele. Alternatively wild type allele lost first followed by duplication of hyperactive allele. Third possibility is recombination events between portions of chromosome 5.

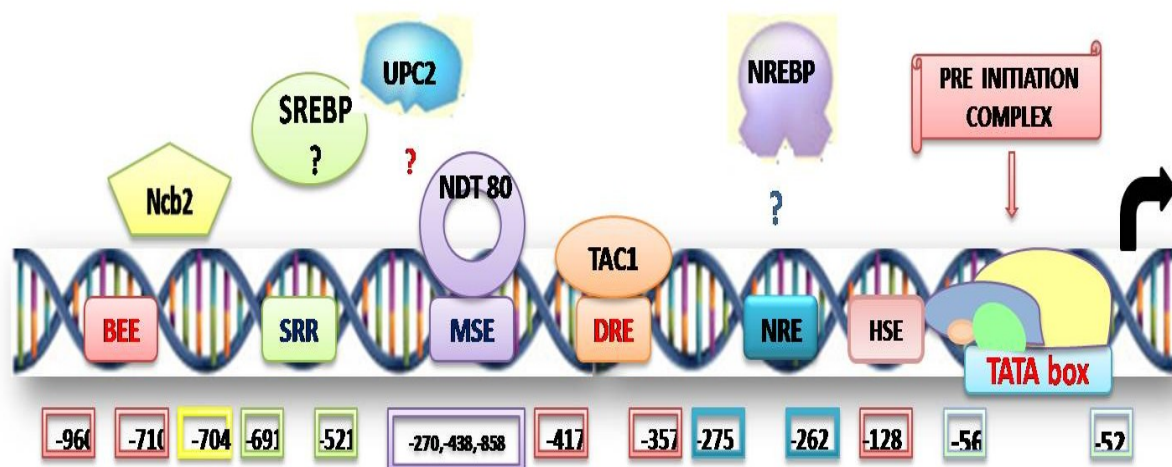


Fig.1 Schematic representation of *Cis* and *Trans* acting elements of *CDR1* promoter of *C.albicans* regulation playing important role in *CDR1* expression

Post transcriptional modification: Post transcription modification also contributes to *CDR1* regulation in *C.albicans*. Enhanced transcriptional activation, increased mRNA stability contributes to *CDR1* over expression in AR isolates. Poly (A) tail is a major determinant of mRNA stability. Increase length of poly (A) tail provides higher stability and efficient translation of transcripts (Manoharlal R et al, 2010). In *C.albicans* PAT assay shown that in AR isolates *CDR1* mRNA has ~30 -35 % longer poly (A) tail as compared to AS isolates. In this report it was shown that Loss of homozygosity at *PAP1* locus is responsible for hyperadenylation and increased stability of *CDR1* transcript and this mechanism is responsible for over expression of *CDR1* in AR isolates (Manoharlal R et al, 2010).

Transcriptional complex positional shift: Mobilization of transcriptional complex away from Transcription start site (TSS) is well established mechanism of gene regulation in higher eukaryotes. In human cells it was shown that *NC2* mobilizes *TBP* upstream from TSS. In

C.albicans it was shown that *Ncb2* mobilize transcription complex away from TSS in upstream direction. Expression of *Ncb2* remains similar in both AS and AR strain but occupancy of *Ncb2* at *CDR1* promoter is different in both AS and AR strain. In AR strain occupancy of *Ncb2* is at TSS. Formation of Transcription complex at TSS facilitates proper transcription and helps in over expression of *CDR1* in AR isolates. (Shukla S et al, 2011) Beside this in AR isolates it was found that *Ncb2* mobilizes a fraction of transcription complex upstream to TSS. It was therefore hypothesized that shifting of transcription complex away from TSS is responsible for low expression of *CDR1* in AS isolates.

Gene looping: In higher eukaryotes and other yeast species it was shown that gene looping is a prominent mechanism of regulation. (Laine JP et al, 2009). In *S. cerevisiae* it was shown that *SSU72*, *GAL10* gene is regulates through looping mechanism. (Ghazy et al, 2009). Gene looping in *Candida albicans* is not yet reported but highly AT rich promoter facilitates gene looping and interestingly

it was found that CDR1 and other Tac1 regulon genes have high AT rich promoter. Further study is required to prove that Gene looping may be a mechanism for regulation of CDR1 and other genes in *C.albicans*.

In conclusion, this review we emphasis on the cis, trans acting factor which regulates the CDR1 expression and molecular mechanisms through which CDR1 expression controlled. CDR1 regulation is a complex process involving a variety of factors. Recently used Genome wide location analysis technique is helpful to recognize the target of a particular transcription factor and the overlap between the targets of two transcription factors. These types of study will give us a new insight of CDR1 regulation. To get insight in to mechanism of CDR1 regulation modern techniques like 3C (chromosomal conformational capture) will be helpful to get new insight in transcriptional mechanism of CDR1. 3C technique will help to find physical interaction between various CDR1 regulators in different conditions like stress, transient drug exposure or in drug resistant isolate will give us a detail picture of CDR1 regulation.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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