SECTION-II
4. Morphogenic regulator EFG1 modulates drug susceptibilities in C. albicans

4.1 Background
Although, mechanisms of antifungal resistance and major factors which contribute to it are fairly established, there are evidences to suggest that MDR is a multi-factorial phenomenon which may be contributed by yet unknown mechanisms. For example, levels of a homolog of bacterial two-component response regulators SSK1 and iron display enhanced sensitivity to drugs in Candida cells (Ruprich-Robert et al., 2008; Prasad et al., 2006). Additionally, there are azoles resistant clinical isolates of C. albicans where mechanisms of resistance appear to be different than the commonly known strategies adopted by Candida (White et al., 1998).

Morphogenic transitions in C. albicans from the unicellular yeast form into filamentous form is an important attribute towards the virulence of this pathogen (as already reviewed in previous section). The transcription factor Efg1p regulates morphogenesis of C. albicans since it induces the yeast-to-hyphal transition and also regulates phenotypic switching and chlamydomspore formation of this pathogen (Sonneborn et al., 1999a; Sonneborn et al., 1999b). Interestingly, iron deprivation induced hyphal morphogenesis in C. albicans is also mediated through transcriptional regulator Efg1p (Hameed et al., 2008).

Considering the importance of Efg1p regulator in morphogenesis and iron also found to be a novel regulator Efg1p (as described in previous section), in this part of the study, it has been evaluated if defects in morphogenic signaling pathways would also affect MDR status of C. albicans cells. It was observed that Δefg1 mutant cells are selectively sensitive to azoles and polyenes as compared to the wild-type (WT) strain when grown on solid agar media. The Δefg1 mutant cells display increased membrane fluidity leading to enhanced passive diffusion of drugs. Taken together, this part of the study establishes a convergence of EFG1 and MDR pathways and thus proposing an additional new role to this important morphogenic regulator of C. albicans.
4.2 RESULTS

4.2.1 Δefg1 mutant shows enhanced susceptibility to drugs

In this study, it has been explored if a link exists between the morphogenic regulator EFG1 and MDR regulatory pathways. For this, Δefg1 mutants was exploited which exhibited defective morphogenesis under various hyphae inducing conditions (Ernst, 2000) and tested their susceptibility pattern to different drugs. For this, two independent methods, namely, spot and filter disc assays were used to evaluate drug susceptibilities of C. albicans cells on solid growth media. The drug testing, which was done by serial dilution spot assays, revealed that there was a marked increase in the susceptibility of the Δefg1 mutant to azoles such as FLC, KTC and ITR, which target lanosterol 14α-demethylase (Fig. 14A). The Δefg1 mutant also displayed enhanced sensitivity to polyenes such as AMB and NYS (Fig. 14A). The sensitivity to many other classes of drugs such as CYH, MTX, CV, PHE, and SMM, remained unaffected in Δefg1 mutant cells (Fig. 14A). The increase in drug sensitivity was highest in the homozygous Δefg1 (Δefg1/Δefg1) mutant cells as compared to heterozygous (Δefg1/EFG1) and revertant (Δefg1/ Δefg1[EFG1]) strains. Notably, the liquid growth media based NCCLS method of MIC determination revealed no change in MIC values of Δefg1 mutant when tested with the same drugs as those used for solid assays (Table 6).
Fig. 14A. Drug resistance profiling of CAF2-1 (WT), BCa09-01 (heterozygous Δefg1/EFG1), HLC52 (homozygous Δefg1 mutant) and HLC74 (retransformed Δefg1/efg1[EFG1]) cells as determined by drug dilution spot assay described in Material and Methods. Drugs were used at the following concentrations: FLC (1 μg/ml), ITR (0.25 μg/ml), KTC (0.05 μg/ml), NYS (1.3 μg/ml), AMB (0.3 μg/ml), CYC (0.1 μg/ml), MTX (1 μg/ml), CV (0.1 μg/ml), O-PHE (2 μg/ml) and SMM (1 μg/ml).
The spot assays were validated by filter disc assay which indicated greater diameter of zone of inhibition with azoles and polyenes for the Δefg1 mutant cells (Fig. 14B).

**Fig. 14B.** Drug resistance profiling of CAF2-1 (WT), BCA09-01 (heterozygous Δefg1/EFG1), HLC52 (homozygous Δefg1 mutant) and HLC74 (retransformed Δefg1/efg1[EFG1]) cells as determined by Filter disc assay described in Materials and Methods. Drugs were used in the following amounts: FLC (32μg); KTC (8μg); ITR (20μg); NYS (10μg); AMB (10μg); CYC (0.5μg); MTX (50μg); CV (5μg); O-PHE (20μg) and SMM (5μg). The clear zones represent the zone of inhibitions in presence of drugs after incubation at 30°C for 48 hours.
Table: 6. Determination of dose ranging inhibition by broth microdilution assay of \( \Delta efg1 \) mutants. The MIC\(_{80} \) (defined as the lowest drug concentration that gave >80 % inhibition of growth as compared to the drug free control) was determined using broth microdilution assay and evaluated both visually and by reading the absorbance at \( A_{600 \text{ nm}} \) in a microtitre plate reader as described in Material and Methods.

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>WT</th>
<th>( \Delta efg1/\text{EFG1} )</th>
<th>( \Delta efg1/\Delta efg1 )</th>
<th>( \Delta efg1/\text{efg1[EFG1]} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLC</td>
<td>1-0.5</td>
<td>1-0.5</td>
<td>1-0.5</td>
<td>1-0.5</td>
</tr>
<tr>
<td>KTC</td>
<td>0.08-0.04</td>
<td>0.08-0.04</td>
<td>0.08-0.04</td>
<td>0.08-0.04</td>
</tr>
<tr>
<td>ITR</td>
<td>0.08-0.04</td>
<td>0.08-0.04</td>
<td>0.08-0.04</td>
<td>0.08-0.04</td>
</tr>
<tr>
<td>AMB</td>
<td>1.25-0.625</td>
<td>0.3125</td>
<td>0.3125</td>
<td>1.25-0.625</td>
</tr>
<tr>
<td>NYS</td>
<td>1.25-0.625</td>
<td>0.3125</td>
<td>0.3125</td>
<td>1.25-0.625</td>
</tr>
<tr>
<td>CV</td>
<td>5-2.5</td>
<td>5-2.5</td>
<td>5-2.5</td>
<td>5-2.5</td>
</tr>
<tr>
<td>SMM</td>
<td>15.63</td>
<td>7.82</td>
<td>7.82</td>
<td>15.63-7.82</td>
</tr>
<tr>
<td>PHE</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MTX</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
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<tr>
<td>CYH</td>
<td>625</td>
<td>625</td>
<td>312.5</td>
<td>312.5</td>
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</table>
4.2.2 Ergosterol levels of \( \Delta \text{efg}1 \) mutant are decreased

Considering the fact that Efg1p affects susceptibilities towards selective class of drugs azoles and polyenes and ergosterol being an important component of membrane, the level of ergosterol was estimated in \( \Delta \text{efg}1 \) mutant. It was observed that ergosterol levels were down (~24%) in the \( \Delta \text{efg}1 \) mutant (Fig. 15). Northern blot results revealed that the \( \Delta \text{efg}1 \) mutant when grown on solid media, display down regulation of \( \text{ERG}11 \) and a simultaneous up regulation of \( \text{ERG}3 \) genes (Fig. 17).

![Ergosterol content graph](image)

**Fig. 15.** Relative percentages of ergosterol content in the control strain CAF2-1 (WT) and HLC52 (\( \Delta \text{efg}1 \) mutant) cells, calculated as described in the Materials and Methods. The values are the mean ± S.D. of more than three independent sets of experiments.
4.2.3 Oleic acid levels are enhanced in Δefg1 mutant

Evaluation for the status of fatty acids (FAs) in the Δefg1 mutant grown on solid media was also done (Table 7). The results show that in the Δefg1 mutant, the content of 18:1 unsaturated oleic acid was significantly increased from 32.8 to 44.6%, while the content of FAs 18:2 and 18:3 were reduced (Table 7). Interestingly, the Δefg1 mutant cells showed considerable up regulation of OLE1 gene encoding fatty acid desaturase (steroyl CoA desaturase) which synthesizes oleic acid (Fig. 17).

**TABLE 7.** Relative percentages of fatty acids in the control strain CAF2-1 (WT) and Δefg1 mutant (HLC52) calculated as described in the Materials and Methods.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>WT</th>
<th>HLC52</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₄:₀</td>
<td>1.3 ± 0.03</td>
<td>1.2 ± 0.02</td>
</tr>
<tr>
<td>C₁₆:₀</td>
<td>20.2 ± 0.9</td>
<td>25 ± 0.99</td>
</tr>
<tr>
<td>C₁₆:₁</td>
<td>11.4 ± 0.2</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>C₁₈:₀</td>
<td>4.7 ± 0.11</td>
<td>3.1 ± 0.10</td>
</tr>
<tr>
<td>C₁₈:₁</td>
<td>32.8 ± 0.83</td>
<td>44.6 ± 0.98</td>
</tr>
<tr>
<td>C₁₈:₂</td>
<td>15.4 ± 0.09</td>
<td>11.2 ± 0.09</td>
</tr>
<tr>
<td>C₁₈:₃</td>
<td>2.3 ± 0.04</td>
<td>1.1 ± 0.02</td>
</tr>
</tbody>
</table>

# The values are the mean ± S.D. of more than three independent sets of experiments.
4.2.4 \(\Delta efg1\) mutant show enhanced membrane fluidity and diffusion of the drug

It was examined whether the observed changes in oleic acid and ergosterol levels affect the physical state of the membrane. The membrane order of cells grown on an agar surface was determined by the mobility of the fluorochrome, 1,6-diphenyl-1,3,5-hexatriene (DPH), using fluorescence polarization measurements (Mukhopadhyay et al., 2002; Prasad et al., 2006). The lower polarization p value implies higher fluorochrome mobility and high membrane fluidity, whereas elevated p values indicate decrease in membrane fluidity. As evident from the observed decrease in “p” values (~43%) the \(\Delta efg1\) mutant membranes were more fluid as compared to WT cells (Fig. 16A).

![Fluidity Graph](image)

Fig. 16A. Fluorescence polarization 'p values' of the control strain CAF2-1 (WT) and HLC52 (\(\Delta efg1\) mutant) cells (x-axis), calculated as described in the Materials and Methods. Mean fluorescence polarization “p” values of the cells ± S.D. of three independent sets of experiments as depicted on y-axis.
In the following experiment, the consequence of enhanced membrane fluidity was examined by monitoring passive diffusion of the drug. The contribution of the efflux pumps was blocked by de-energizing the cells to deplete the intracellular ATP (Mukhopadhyay et al., 2002; Prasad et al., 2006) and passive diffusion was then monitored in the de-energized cells by using two drugs such as R6G and radio labeled $[^3]$H-FLC (Mukhopadhyay et al., 2002; Prasad et al., 2006). Fig. 16B & C depicts extracellular levels of R6G and intracellular levels of $[^3]$H-FLC (accumulation), in the $\Delta.efg1$ mutant as compared to the WT cells. The disruption of $EFG1$ resulted in enhanced passive diffusion as was evident from decrease (~26%) in the extracellular concentration of R6G and increase (~41%) in intracellular accumulation of $[^3]$H-FLC (Fig. 16B & C).

![Graph](image)

Fig. 16B. Passive diffusion of R6G in the control strain CAF2-1 (WT) and HLC52 ($\Delta.efg1$ mutant) cells (x-axis), calculated as described in the Materials and Methods. Mean of the OD$_{527}$ of the supernatant ± S.D. of three independent sets of experiments as depicted on y-axis.
Fig. 16C. Passive diffusion of $[^3]$H-Fluconazole in the control strain CAF2-1 (WT) and HLC52 (∆efg1 mutant) cells (x-axis), calculated as described in the Materials and Methods. Mean of the intracellular concentration of $[^3]$H-Fluconazole ± S.D. of three sets of experiments as depicted on y-axis.
4.2.5 Enhanced Drug susceptibility of Δefg1 mutant is independent of multidrug efflux transporters

Over expression of drug efflux pumps is one of the well-known mechanisms for the development of resistance in *C. albicans* (Albertson *et al.*, 1996; Ben-Yaacov *et al.*, 1994; Gupta *et al.*, 1998; Kohli *et al.*, 2002; Pao *et al.*, 1998; Sanglard *et al.*, 1997). To examine the role, if any, of major ABC efflux pumps like *CaCDR1*, *CaCDR2* and MFS pumps like *CaMDR1*, in synergism with *EFG1* disruption and drug sensitivity, Northern Blottings were employed. The mRNA levels were checked for the genes encoding major drug efflux transporters namely *CaCDR1*, *CaCDR2* and *CaMDR1* and found no considerable change in the transcript levels of these genes (Fig. 17).

![Northern blot analysis](image)

**Fig. 17.** Northern blot analysis of *OLE1, ERG3, ERG11, CDR1, CDR2* and *CaMDR1* in the control strain CAF2-1 (WT) and HLC52 (Δefg1 mutant) cells. Left panels show transcript levels in lanes (1) WT, (2) HLC52. The panel on the right side next to each of the transcript represents loading control for indicating equal gel loading of total RNA for the respective gene transcripts.
4.2.6 Endogenous ROS levels is enhanced in Δefg1 mutant

ROS elevation is associated with many metabolic statuses of Candida cells (Vinolo et al., 2009). We explored if EFG1 modulates ROS levels and affect drug susceptibilities. For this, alteration in ROS production was measured by DCFDA fluorescence method where increase fluorescence within cells implies enhanced ROS levels, which is depicted as relative fluorescence value (Rf). Interestingly, the results demonstrated that the endogenous levels of ROS were considerably elevated (~54%) in Δefg1 mutant as compared to the WT cells (Fig. 18A & B). Expectedly, the enhanced ROS levels could be reversed if media was supplemented with natural as well as synthetic antioxidants such as AA and PDTC respectively (Fig. 18A & B). However, the restoration of ROS levels by antioxidants did not revert drug sensitivity of Δefg1 mutant cells (Fig. 18C). Interestingly, the transcript levels of genes detoxifying the ROS levels in Candida cells were also found to be considerably down regulated in Δefg1 mutant cells (Fig. 18D).

![Fig. 18A. ROS production in the control strain CAF2-1 (WT) and HLC52 (Δefg1 mutant) cells were measured by DCFDA fluorescence method as described in Materials and Method. Upper panel depicts the fluorescence intensity (higher fluorescence depicts higher ROS production) as measured by fluorescence microscope of WT, Δefg1 and Δefg1 treated with antioxidants viz. AA and PDTC with final concentrations 5mM and 10μM respectively. Lower panel depicts the phase contrast micrographs of the upper panel. (Magnification 63X)
Fig. 18B. Quantitative measurement of ROS production in the control strain CAF2-1 (WT) and HLC52 (Δefg1 mutant) cells. ROS levels in WT, Δefg1 and Δefg1 treated with antioxidants viz. AA (5mM) and PDTC (10μM) respectively depicted in x-axis as measured by Relative Fluorescence (Rf) values. Mean of the Relative Fluorescence (Rf) values ± S.D. of the three sets of experiments as depicted on y-axis.
Fig. 18C. Drug resistance profiling of CAF2-1 (WT) and HLC52 (Δefg1 mutant) cells determined by drug dilution spot assay in presence of AA (5mM) and PDTC (10μM) as described in Material and Methods. Drug was used at the following concentration: FLC (1 μg/ml).
Fig. 18D. Northern blot analysis of RBT5, YHB1, SOD2, CDC19, CTA1, DDR48, GRP2 in the control strain CAF2-1 (WT) and HLC52 (Aefg1 mutant) cells. Left panels show transcript levels in lanes (1) WT, (2) HLC52. The panel on the right side next to each of the transcript represents loading control for indicating equal gel loading of total RNA for the respective gene transcripts.
4.2.7 Iron deprivation enhances drug sensitivity of Δefg1 mutant

It has been earlier observed that iron deprivation of C. albicans cells lead to increase in drug sensitivity (Prasad et al., 2006). Here it was observed that Efg1p levels affect drug susceptibilities in C. albicans as Δefg1 null mutant cells became sensitive to drugs, particularly those targeting ergosterol or its metabolism. With this background, it was evaluated if iron depletion in Δefg1 could lead to any further change in drug susceptibility of C. albicans cells. For this, two independent methods of drug susceptibility testing were used, namely broth microdilution and spot assays (Fig. 19A and B). Both the methods confirmed our previous results (Lan et al. 2004), that the cells growing in IPM were distinctly more susceptible to FLC (MIC$_{80}$ values 0.125 μg/ml) as compared to those growing in ISM (MIC$_{80}$ values 0.5 μg/ml) (Fig. 19B). Spot assays also confirmed MIC results obtained from broth microdilution assay (Fig. 19A). As reported earlier, the Δefg1 null mutant cells on spot assay showed enhanced sensitivity to FLC. However, this sensitivity of Δefg1 null mutant cells was further enhanced if mutant cells were deprived of iron.
Fig. 19A Drug resistance profile of $\Delta efg1$ cells of Candida albicans as determined by spot assay for FLC in the absence and presence of 150 μM BPS. For spot assays, five microlitres of five fold serial dilutions of each yeast culture ($A_{600}$, 0.1) was spotted on to YEPD plates in the absence (control) and presence of FLC (2 μg/ml). Growth differences were evaluated following incubation of the plates for 48 hours.

Fig. 19B Determination of dose ranging inhibition by broth microdilution assay of $\Delta efg1$ cells with fluconazole (FLC) in concentration varying from 0.13 to 64 μg/ml in the absence (●, filled diamond) and presence (□, open square) of 150 μM BPS. The MIC$_{90}$ (defined as the lowest drug concentration that gave >80 % inhibition of growth as compared to the drug free control) was determined using broth microdilution assay and evaluated both visually and by reading the absorbance at $A_{600}$ nm in a microtitre plate reader as described in Material and Methods.
4.3 Discussion

In this part of the study, it is reported that disruption of the morphogenic regulator \( EFG1 \) enhances drug sensitivity of \( C. \) \textit{albicans} cells by a mechanism that is not dependent on the drug efflux pumps. Efg1p disruption showed a gene dosage effect on enhanced susceptibilities to drugs which was highest in homozygous mutant (\( \Delta efg1/\Delta efg1 \)) as compared to the wild type, heterozygous (\( \Delta efg1/EFG1 \)) and revertant (\( \Delta efg1/\Delta efg1[EFG1] \)) strains. It was shown that \( \Delta efg1 \) mutant displayed sensitivity to azoles (FLC, ITR, KTC) and polyenes (AMB and NYS) when grown on agar surface (Fig. 14A & B). Interestingly, the increased drug susceptibility phenotype was selective since sensitivity to many other classes of tested drugs such as CYH, MTX CV, PHE and SMM remained unaffected in \( \Delta efg1 \) mutant (Fig. 14A & B). Notably, the observed enhanced susceptibility of \( \Delta efg1 \) mutant was only confined to cells when grown on solid agar surface and not when grown in liquid media (Table 6). It is reported by Ernst group that the \( \Delta efg1 \) fail to form hyphae when exposed to inducers such as serum or \( N \)-acetyl-\( D \)-glucosamine (GlcNAc) in liquid or solid media, however, under microaerophilic/embedded conditions, \( \Delta efg1 \) mutant displays enhanced filamentous growth (Brown \& Gow, 1999; Doedt \textit{et al.}, 2004; Setiadi \textit{et al.}, 2006). This establishes the dual role of Efg1p regulator in controlling the same phenomenon of hyphal morphogenesis via different regulatory mechanisms owing to different growth conditions. Therefore, confinement of drug sensitivity of \( \Delta efg1 \) mutant cells, grown only on solid agar surface does not reflect an art factual effect due to difference in growth media. This was further validated by the fact that drug susceptibility assays which were performed in solid surface \textit{via} two independent methods, gave similar phenotype (Fig. 14A & B). Notably, \( C. \) \textit{albicans} cells form biofilms on plastics and host of biological surfaces which show elevated antifungal resistance (Mukherjee \textit{et al.}, 2005) and pose a major challenge for antifungal chemotherapy. Since Efg1p levels are pivotal for the biofilm formation (Ramage \textit{et al.}, 2002), its role in antifungal resistance during surface growth may be of particular clinical relevance.
This study supports that the MDR phenomenon in yeast is the result of culmination of several factors. Notwithstanding the involvement of any known MDR genes, \( \Delta efg1 \) mutant cells consistently showed decreased resistance on solid growth media to a restricted class of drugs targeting ergosterol or its metabolism. Ergosterol is an essential and specific component of fungal membranes performing variety of functions that modulate membrane fluidity and its permeability (Daum et al., 1998). Therefore, transcription factors regulating the ergosterol biosynthetic pathway represents an important target for antifungal agents. Interestingly, in an earlier study the transcript level of \( ERG3 \) has been shown to be directly affected by the \( EFG1 \) levels (Lo et al., 2005). In this study, a considerable down regulated transcript level of \( ERG11 \) (the rate limiting step in ergosterol biosynthesis) and up-regulated \( ERG3 \) transcript was observed in \( \Delta efg1 \) mutant in comparison to the WT cells (Fig. 17). This was associated with a statistically significant decrease in ergosterol contents (~24%) in \( \Delta efg1 \) mutant cells (Fig. 15). Of note, \( ERG3 \) acts downstream of \( ERG11 \) in the ergosterol biosynthesis pathway and encodes the \( \Delta^{5,6} \)-desaturase (Chau et al., 2005). In an azole-inhibited pathway, Erg3p is responsible for converting the nontoxic 14-methyl intermediates (Chau et al., 2005) into the toxic sterol 14-methylergosta-8,24(28)-dien-3,6-diol. There have been reports (Kelly et al., 1997; Kakeya et al., 2003) which demonstrate that inhibition of 14 alpha-demethylase by azoles results not only in ergosterol depletion but also in the accumulation of methylated sterol 14 alpha-methylergosta-8, 22 (28)-dien-3 betas, 6 alfa-diol. Moreover, even deletion of \( ERG3 \) gene, in \( C. albicans \), resulted in reduced susceptibility of \( \Delta erg3 \) to azoles (Kakeya et al., 2003) and amphotericin B (Kelly et al., 1997). Sterol analysis revealed that \( \Delta erg3 \) mutant lost both ergosterol and toxic diol product when grown in presence of azoles (Kelly et al., 1997; Kakeya et al., 2003). In the present study, in \( \Delta efg1 \) mutant cells where \( ERG3 \) is up-regulated, azoles might act synergistically due to the accumulation of the toxic diol product, however, this remains to be experimentally validated.

Considering the fact that sensitivity to the azoles has often been linked to the changes in membrane lipid composition (Kohli et al., 2002; Mukhopadhyay et al., 2002; Prasad et
al., 2006) and that the enhanced drug sensitivity phenotype of ΔefgI mutant cells observed presently was restricted only to azoles and polyenes, it was speculated that probably EFG1 target membrane-associated genes, which affect its composition. Our Northern blot analysis revealed a marked increase in OLE1 transcript in ΔefgI mutant (Fig. 4) in comparison to the WT cells coinciding with a significant increase in the oleic acid content of the membrane (Table 7) leading to an increase in membrane fluidity (Fig. 16A). Although it is expected that normally cells would cope with the fluctuations in oleic acid levels by compensatory adjustments in membrane lipids, which would maintain optimal membrane fluidity. The observed increase in membrane fluidity implies a direct link between Olelp and Efglp levels. Earlier, it has been shown that fatty acid biosynthesis genes require Efglp and particularly, fatty acid unsaturation in C. albicans is dependent on Efglp levels under hypoxic conditions (Setiadi et al., 2006). In the present study, a direct functional consequence of oleic acid increase was observed as an increase in membrane fluidity of ΔefgI mutant cells. Consequently, the passive diffusion of drugs is increased in ΔefgI mutant cells which resulted in enhanced accumulation and sensitivity of drugs (Fig. 16B & C).

Among novel mechanisms of drug resistance, role of ROS is emerging where increase in ROS levels has been associated with several antifungals (Kobayashi et al., 2002). Interestingly, ΔefgI mutant cells displayed enhanced levels of endogenous ROS (Fig. 18A & B) which coincided with the down regulation of many of the genes involved in ROS detoxification (Fig. 18D). However, the enhanced ROS levels which could be reversed by the addition of antioxidants (Fig. 18A & B), could not reverse drug sensitivity of ΔefgI mutant cells (Fig. 18C). There are reports to suggest that Candida strains lacking ERG11 are susceptible to oxidative stress (Kan et al., 1996). It could, therefore be possible that the inhibition of ERG11 gene by EFG1 may produce similar effect. Whether increased ROS levels in ΔefgI mutant acts as a signal for some regulatory cascade acting in parallel or in concert with the drug susceptibility mechanisms remains an open area of interest.
Taken together, this study indicates that apart from the well established role of EFG1 as morphogenetic regulator, it also functions as a regulator of antifungal resistance. The results revealed that increased membrane fluidity leading to enhanced passive diffusion of drugs could be critical determinants of drug susceptibilities of the Δefg1 mutant cells. Probably, the regulatory networks which control the morphogenesis in C. albicans on solid media also control genes involved in cellular functions not directly related to drug resistance. Certainly more extensive analyses are required to elucidate the commonality between EFG1 and MDR signaling cascades to find newer targets for antifungal chemotherapy.

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