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**YAP1** confers resistance to the fatty acid synthase inhibitor cerulenin through the transporter Flr1p in *Saccharomyces cerevisiae*

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**Abstract**

In this study, we utilized a genetic approach to identify genes which render yeast cells resistant to cerulenin (Cer), a potent and noncompetitive inhibitor of fatty acid synthase (FAS). Overexpression of the yeast transcription factor Yap1p was found to confer Cer resistance (CerR). This resistance was shown to be less pronounced in a strain deleted for YCF1, a multidrug resistance ABC transporter, supporting previous observations that implicated YCF1 in mediating CerR. However, isolation of YAPI as a high-copy CerR gene in a ycf1Δ strain suggested that YAPI-induced CerR was mediated by additional downstream effectors. Overexpression of neither glutathione reductase nor a predicted aryl alcohol dehydrogenase (the products of two YAPI-regulated genes involved in detoxification) conferred CerR. Overexpression of ATR1, another YAPI-regulated gene previously implicated in conferring resistance to a number of cytotoxic drugs, was also incapable of making cells resistant to Cer. In contrast, overexpression of Flr1p, a yeast transporter of the major facilitator superfamily which is also under the control of YAPI, was sufficient to confer CerR in an otherwise wild-type background. Moreover, CerR was markedly diminished in a strain deleted for FLR1. These findings implicate members of both of the transporter superfamilies involved in multiple drug resistance (MDR) in the acquisition of CerR in yeast. Furthermore, our studies indicate that yeast may be a useful model system in which to investigate the role of FAS in cancer biology and the effects of Cer on eukaryotic cell growth.

**Key words**

Yeast · Cerulenin resistance (CerR) · **YAPI** · FLR1 · YCF1

**Introduction**

Overexpression of fatty acid synthase has been identified as a marker of poor prognosis in breast cancer (Kuhajda et al. 1994). An increase in the levels of endogenous fatty acid synthesis has been demonstrated to accompany overexpression of FAS in a number of established human breast carcinoma cell lines, suggesting that FAS activity parallels FAS expression in these cell lines. Furthermore, cerulenin, a potent and noncompetitive inhibitor of FAS, has been found to induce programmed cell death in FAS-overexpressing human breast cancer cell lines, indicating that FAS activity may be essential for the aggressive growth characteristics of these cell lines (Pizer et al. 1996a). A recent study using a xenograft model of ovarian cancer in nude mice has shown that treatment with Cer and consequent inhibition of FAS activity results in regression of established ascites tumors, a reduction in the incidence of ascites and a significant increase in survival (Pizer et al. 1996b). The human promyelocytic leukemia cell line HL60 also expresses elevated levels of FAS, demonstrates increased fatty acid synthesis and is markedly growth-inhibited by Cer, indicating that the chemotherapeutic potential of Cer may not be limited to the treatment of epithelial carcinomas (Pizer et al. 1996c).

FAS activity is also vital to the normal growth of microorganisms including yeast, fungi and bacteria, as demonstrated by the potent antibiotic effects of Cer (Omura 1976; Saermark et al. 1991; Duronio et al. 1992; Johnson et al. 1994a,b; Saitoh et al. 1996; Sanglard et al. 1996). Such microorganisms may provide useful model systems in which to investigate the uptake, metabolism
and toxicity of, and resistance to, this potential chemotherapeutic agent. To this end, we have utilized a genetic approach in yeast to identify genes which confer resistance to cerulenin (CerR). Overexpression of the Jun-like transcription factor Yap1p, which functions as a multidrug resistance and stress-response regulator in yeast (Rowley et al. 1988, 1989; Gounalaki and Theirotos 1994), was found to confer CerR. Furthermore, this resistance was independent of YCF1, a gene encoding a multidrug resistance ABC transporter, which was previously shown to be under the control of YAP1 (Wennie et al. 1994b). We also investigated other potential downstream mediators of YAP1-induced cerulenin resistance. These studies have identified Fert1p as a second yeast drug transporter involved in mediating resistance to cerulenin. Our findings implicate both major MDR yeast drug transporter involved in mediating resistance in yeast, and indicate that yeast may be a useful model system in which to pursue the role of FAS in cancer biology and the effects of Cer on eukaryotic cell growth.

Materials and methods

Yeasts strains and growth conditions

The Saccharomyces cerevisiae strain JK93d (Heitman et al. 1991; ura3-52 leu2-3, -112 his3-A200 trp1-A901 lys2-801 suc2-A9 Me+) and the ycf deletion strain JYW53 (MATa leu2-3,-112 ura3-52 his3-A200 trp1-A901 lys2-801 suc2-A9 Me+) were provided by Dr. W. Scott Moyle-Wemmel (Wemmen et al. 1994a). The strains MRY1-3A (ade2 his3 leu2 trp1 ura3 can1 FLR1) and MRY1-3B (ade2 his3 leu2 trp1 ura3 can1 fyr1::HIS3), transformed with YpEp352 (a URA3-based, multicopy vector) alone or with pYEPI52-YAPI, were provided by Dr. Martine Raymond (Alarco et al. 1997). Cerulinin (Sigma) from a 5 mg/ml stock solution in DMSO was diluted to a final concentration of 20 μg/ml in YEPD medium (10 g yeast extract, 20 g peptone, 20 g bacto-agar, 20 g dextrose per l) for the preparation of selective plates. Where indicated, galactose was substituted for glucose as a carbon source in Cer-containing plates, to allow induction of genes under control of the GAL promoter.

Plasmids

A yeast genomic library constructed in pRS202 was kindly provided to us by P. Hieter (unpublished). The vector is a modified version of the pRS306 vector, and carries a 2 μ origin of replication. A partial Sac3A1 digest of yeast genomic DNA was size fractionated and inserted into BamHI/BglII-digested pRS202, giving rise to inserts of approximately 6–8 kb in length (Sikorski and Hieter 1989). Wild-type yeast cells were transformed with this library and selected for uracil prototrophy, and transformants were then pooled and replated onto Cer containing plates. The pYES2 yeast expression vector (Invitrogen) was used for regulatable expression of YAPI. This vector contains the URA3 gene (which allows transformants to grow in media without uracil), and an ampicillin resistance marker and origin of replication that function in E. coli. Genes expressed using this system are placed under the control of the GAL promoter, which allows expression in the presence of galactose and not in the presence of glucose. The YpEp352 vector is a URA3-based, multicopy vector (Alarco et al. 1997). Yeast transformations were performed using the lithium acetate method, as previously described (Ito et al. 1983).

Molecular biological methods

Restriction enzymes and Vent polymerase were from New England Biolabs, and were used according to the manufacturer’s recommendations. DNA sequence analysis was performed by the method of Sanger et al. (1977) using a Sequenase kit from United States Biochemicals. Isolation of plasmids from yeast was performed as follows: 2 ml of overnight culture was centrifuged at 5000 rpm for 5 min, and the cells resuspended in 100 μl of STET buffer (2% sucrose, 5% Triton X-100, 50 mM TRIS-HCl, 50 mM EDTA pH 8), to which 0.2 g of glass beads was added. Cells were vortexed for 5 min at 4°C, followed by the addition of 100 μl of STET buffer and boiling for 3 min. After briefly cooling on ice and centrifugation at 13,000 rpm for 10 min at 4°C, 100 μl of the supernatant was added to 50 μl of 7.5 M ammonium acetate, and genomic DNA was allowed to precipitate to −20°C for 2 h. After a 10-min centrifugation at 13,000 rpm, 100 μl of the supernatant was added to 200 μl of ice-cold ethanol and the precipitated plasmid DNA was washed with 70% ethanol, dried and resuspended in 20 μl of sterile water for use in E. coli transformations. Transformation of E. coli was performed by electroporation of competent cells using a Bio-Rad E. coli Pulser according to the manufacturer’s recommendations.

Cloning of yeast genes

All of the genes studied here (unless otherwise indicated) were cloned downstream of the GAL promoter in pYES2 (Invitrogen). The cloning strategy involved PCR amplification of the gene of interest using high-fidelity Vent DNA polymerase (New England Biolabs), simultaneously introducing appropriate restriction enzyme sites in the flanking regions. All oligonucleotides were synthesized by Operon Technologies.

Results

Characterization of a cerulenin resistance gene

To determine the toxicity of cerulenin to S. cerevisiae cells growing on agar plates, 10^6 exponentially growing wild-type yeast cells were inoculated onto YEPD agar plates infused with either 0.2 μg/ml, 2.0 μg/ml or 20 μg/ml Cer. Whereas Cer at 0.2 and 2.0 μg/ml did not significantly affect viability, 20 μg/ml Cer completely abolished growth, and no colonies were observed for as long as 2 weeks (data not shown). This effect was not strain specific, in that strains with three different genetic backgrounds were equally sensitive to 20 μg/ml Cer.

To identify genes which confer CerR, the wild-type strain JK93d was transformed with the yeast genomic library in pRS202. This library is carried on a yeast expression vector which is maintained at high copy number and, thus, should allow identification of genes which provide CerR when overexpressed. Transformants were selected for uracil prototrophy, pooled and replated onto plates containing 20 μg/ml Cer. Screening of approximately 5 × 10^6 transformants yielded about 250 colonies which were resistant to Cer. Serial restreaking of these colonies onto Cer plates identified 12 highly resistant clones, and these were evaluated further.