Two new yeast genes, named MBR1 and MBR3, were isolated as multicopy suppressors of the growth defect of a strain lacking the HAP2 transcriptional activator. Both genes when overexpressed can also suppress the growth defect of hap3 and hap4 null mutants. However, overexpression of MBR1 cannot substitute for the HAP2/3/4 complex in activation of the CYC1 gene. Nucleotide sequencing of MBR1 and MBR3 revealed that these two genes encode serine-rich, hydrophilic proteins with regions of significant homology. The functional importance of one of these conserved regions was shown by mutagenesis. Disruption of MBR1 leads to a partial growth defect on glycerol medium. Disruption of MBR3 has no major effect but the double disruptant shows a synthetic phenotype suggesting that the MBR1 and MBR3 gene products participate in common function.

**Key words** Multicopy suppressors
HAP2/3/4 activation complex
Saccharomyces cerevisiae

**Introduction**

Mitochondria are complex organelles that contain their own genome. This genome encodes only a small fraction of the mitochondrial components. Most of the mitochondrial proteins are encoded by nuclear genes and imported into mitochondria (see Pon and Schatz 1991 for recent review). Two factors are known to have major effects on the regulation of mitochondrial biogenesis: oxygen and carbon source (reviewed in Forsburg and Guarente 1989a; Zitomer and Lowry 1992). Oxygen is required for growth of yeast cells on respiratory substrates (glycerol, ethanol, lactate, etc.) and is known to act via heme as a signal for the regulation of several genes (CYC1, CYC7, CYT1). In these cases, regulation is mediated by the CYP1 (HAP1) transcription activator (Clavillier et al. 1976; Verdière et al. 1986). However, oxygen has also been shown to regulate directly the expression of PET494, which encodes a protein necessary for translation of specific mitochondrial transcripts (Marykwas and Fox 1989). Most of the genes participating in mitochondrial biogenesis are regulated by the carbon source, expression of these genes being repressed in the presence of glucose. The molecular mechanism responsible for glucose repression is complex and not yet totally understood (reviewed in Trumbly 1992). Glucose regulation of many genes encoding subunits of the electron chain complexes is mediated through activation by the HAP2/3/4 complex (see Forsburg and Guarente 1989a for review).

HAP2, HAP3 and HAP4 are components of a protein complex (composed of at least three subunits) that binds to promoter sequences and activates transcription (Forsburg and Guarente 1989a). HAP2, HAP3 and HAP4 participate in the regulation of several genes encoding components of the respiratory chain, Krebs cycle and heme biosynthesis pathway (reviewed in Forsburg and Guarente 1989a; Zitomer and Lowry 1992). HAP2 and HAP4 synthesis is itself regulated by the carbon source at the transcriptional level and therefore control of synthesis of the complex could be the way its activity is regulated in response to carbon source (Pinkham and Guarente 1985; Forsburg and Guarente 1989b). It has been shown that the SSN6 and SNF1 gene products play a role in the glucose repression of COX6 and CYC1 possibly by regulating the synthesis or function of the HAP2/3/4 complex (Wright and Poyton 1990). In the absence of one of the subunits of the HAP2/3/4 complex, yeast strains are unable to grow on medium containing lactate as a carbon source (Pinkham and Guarente 1985; Olesen et al. 1987; Forsburg and Guarente 1989b). This growth defect in a strain carrying a hap2 null allele cannot be reverted suggesting that the
limiting factor for growth on lactate cannot be easily bypassed (Pinkham et al. 1987). These authors proposed that in the absence of HAP2 several genes are underexpressed and become limiting for growth. From the analysis of cytochrome spectra in the hap strains, Mattoon et al. (1990) proposed that cytochrome synthesis is probably not limiting for growth of the hap strains on non-fermentable carbon sources. The physiological reason for the growth defect of the hap strains is still unknown.

In order to elucidate further the physiological role of the HAP2/3/4 complex, we have isolated genes that functionally interact with it. In this paper we present the characterization of two multicopy suppressors of the hap2 growth defect on glycerol medium.

Materials and methods

Media

Yeast rich medium contains 1% yeast extract, 1% Bacto peptone and either 2% glucose, 2% ethanol, 2% galactose or 2% glycerol. Lactate medium is 0.75% yeast extract, 0.75% Bacto peptone, 0.4% NaOH, 4.1% potassium phthalate and 0.41% lactic acid. Yeast rich minimal medium contains 0.17% yeast nitrogen base, 0.5% ammonium sulfate and either 2% glucose or 2% galactose.

Escherichia coli strains

The strains of E. coli used were: DH1 [F−, recA1, gyrA96, endA1, thr-1, hisD17, lacZ17, supE44]; M8820 [F−, araD139, (ara-leu)-7697, (proAB-argF-lacIPOZYA)XIII, rpsL(Sm−)]; JM109 [recA1, (lac-proAB), endA1, gyrA96, thr-1, hisD17, supE44, relA1, F−, traD36, proAB+, lacY1, ZM15]; JM109::(Mucts), as JM109 with a Mucts helper phage integrated in the chromosome.

Yeast strains

The yeasts strains used were: BWG1-7a (MATa, leu2-3, 112, his4-519, ade1-100, ura3-52); J01-1a (MATa, leu2-3, 112, his4-519, ade1-100, ura3-52, hap2); SHY40 (MATa, leu2-3, ade1-100, ura3-52, hap3); Y404 (MATa ade1-100, ura3-52, his4-519, hap4; LEU2); W303 (MATa/a, leu2, ura3, trpl, his3, ade2); W775-2C (leu2, ura3, trpl, his3, ade2); 2CR1A (leu2, ura3, trpl, mbr1-2 ade2); 2CR3 (mbr3-1 ura3, trpl, his3, ade2); R100[yho0] (MATa, ura1, ura2, nsul100, ade2). The Y404, hap4-disrupted, strain was constructed from BWG1-7a as described in Forsburg and Guarente (1989b).

Plasmids

pJP103 (Pinkham and Guarente 1988) is a centromeric plasmid carrying the HAP2 gene and the URA3 marker. pGM51 is a multicopy plasmid (derivative of YEp13; Broach et al. 1979) carrying a 5-kb yeast genomic DNA fragment containing the MBR1 gene. pGM188 is a centromeric derivative of pGM51 constructed by insertion of a BglII fragment containing the MBR1 gene into the vector YCplac111 (Gietz and Sugino 1988). pGM54 was obtained by an EcoRV deletion of the pGM51 plasmid. This deletion removes the 2μ and LEU2 sequences of pGM51 but does not remove insert sequences. pGM189 is a centromeric plasmid (YEp13; Broach et al. 1979) carrying the MBR3 gene (1950 bp HindIII-BamHI fragment) and pGM190 is a centromeric plasmid (YCplac111; Gietz and Sugino 1988) carrying the MBR3 gene.

The plasmid used to construct the MBR3-lacZ fusion, pGM195, was obtained by insertion of a 1950 bp HindIII-BamHI DNA fragment containing the MBR3 gene into pGM194 (a plasmid constructed by ligation of the Scal-PvuII fragment containing the replication origin of pUC19 and the Scal-PvuII fragment containing the tetracycline gene of pBR322).

Transposon

The mini-Mu MudderZ4 is a derivative of MudderZ1 (Daignan-Formier and Bolotin-Fukuhara 1998) in which the two SalI restriction sites surrounding the 2μ replication origin have been replaced by two NotI sites. This new mini-Mu was introduced into a JM109::Mucts strain as described in Daignan-Formier and Bolotin-Fukuhara (1988).

β-Galactosidase (βGal) fusions

Fusions were obtained by transposition of MudderZ4 in plasmids containing the MBR1 and MBR3 genes (pGM54 and pGM195, respectively). Minimuduction was done as described in Daignan-Formier and Bolotin-Fukuhara (1988). One fusion in each gene was used for regulation studies. L15 is a fusion at residue 179 in the MBR1 protein. G1 is a fusion at residue 193 in the MBR3 protein. In vitro βGal assays were done as described in Ruby et al. (1983). The plasmid carrying the CYC1-lacZ fusion, named pLG669Z, is described in Guarente and Mason (1983).

Plasmid manipulation in yeast

Yeast transformation was performed by the lithium acetate method (Ito et al. 1983). Plasmid extraction from yeast was done according to Hoffman and Winston (1987).

DNA analysis

Digestions with restriction enzymes, ligations and E. coli transformation were done according to Maniatis et al. (1982). Nucleotide sequence was determined by the method of Sanger et al. (1977).

Genomic library

Construction of the genomic library from the R100 strain (Contamine and Bolotin-Fukuhara, 1984) was done in the following way. Total DNA from the R100[rho0] strain was extracted and partially digested with Sau3A. DNA fragments of 5 to 10 kb were obtained after separation on a low-melting point agarose gel and inserted into the YEp13 plasmid (Broach et al. 1979), previously cleaved with BamHI and dephosphorylated. After transformation into E. coli DH1, 107 transformants were obtained, 95% of which contained inserted yeast DNA in the YEp13 plasmid.

Gene disruption

The MBR1 gene was interrupted by insertion of a 1.1 kb HindIII DNA fragment containing the URA3 gene at the unique HindIII site (position 473 in Fig. 2) in the sequence of the MBR1 gene (this mutant allele was named mbr1-1). A Xhol (position 243) – EcoRI (position 908) DNA fragment of 1.7 kb containing this construct was used for one-step gene disruption (Rothstein 1983). A deleted allele of MBR1 (called mbr1-2) was also constructed; in this case a fragment of the MBR1 gene (from nucleotide 243 to 1229 in Fig. 2) was replaced by the HIS3 gene. The MBR3 null allele (mbr3-1) was constructed by replacement of a DNA fragment (nucleotides 1053 to 1710 in Fig. 3) at the 3′ end of the MBR3 gene by the LEU2 gene. This construct was inserted at the