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O2R, a Novel Regulatory Element Mediating Rox1p-Independent O_2 and Unsaturated Fatty Acid Repression of *OLE1* in *Saccharomyces cerevisiae*

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Fatty acid desaturation catalyzed by fatty acid desaturases requires molecular oxygen (O_2). *Saccharomyces cerevisiae* cells derepress expression of *OLE1* encoding Δ9 fatty acid desaturase under hypoxic conditions to allow more-efficient use of limited O_2. It has been proposed that aerobic conditions lead to repression of *OLE1* by well-established O_2-responsive repressor Rox1p, since putative binding sequences for Rox1p are present in the promoter of *OLE1*. However, we revealed in this study that disruption of *ROX1* unexpectedly did not affect the O_2 repression of *OLE1*, indicating that a Rox1p-independent novel mechanism operates for this repression. We identified by promoter deletion analysis the 50-bp O_2-regulated (O2R) element in the *OLE1* promoter approximately 360 bp upstream of the start codon. Site-directed mutagenesis of the O2R element showed that the putative binding motif (5′-GATAA-3′) for the GATA family of transcriptional factors is important for O_2 repression. Anaerobic derepression of *OLE1* transcription was repressed by unsaturated fatty acids (UFAs), and interestingly the O2R element was responsible for this UFA repression despite not being included within the fatty acid-regulated (FAR) element previously reported. The fact that such a short 50-bp O2R element responds to both O_2 and UFA signals implies that O_2 and UFA signals merge in the ultimate step of the pathways. We discuss the differential roles of FAR and O2R elements in the transcriptional regulation of *OLE1*.

The lipid composition of cellular membranes is regulated to maintain membrane fluidity (22). A key enzyme involved in this process is the membrane-bound Δ9 fatty acid desaturase, which catalyzes the introduction of the initial double bond between the 9th and 10th carbons of palmitoyl-coenzyme A (CoA) and stearoyl-CoA (35). The correct ratio of saturated to monounsaturated fatty acids contributes to membrane fluidity. Alterations of this ratio have been implicated in various diseases including cardiovascular diseases, obesity, non-insulin-dependent diabetes mellitus, hypertension, neurological diseases, immune disorders, and cancer in mammals (35). In yeast, this ratio has been suggested to be related to the heat shock response, ethanol tolerance, and mitochondrial movement and inheritance (1, 8, 45). The regulation of the expression of Δ9 fatty acid desaturase is, therefore, of considerable physiological importance.

In *Saccharomyces cerevisiae*, Δ9 fatty acid desaturase is encoded by *OLE1* (46). The steady-state level of *OLE1* mRNA is regulated at the level of transcription and by mRNA stability, and both regulatory processes are affected by the presence of unsaturated fatty acids (UFAs) in the growth medium (6, 10, 20). Addition of exogenous UFA represses the transcription of *OLE1* and promotes the decay of *OLE1* mRNA. A fatty acid-regulated (FAR) element which is essential for UFA repression of *OLE1* transcription under aerobic conditions has been identified by promoter deletion analysis (10). Simultaneous disruption of the two long-chain (C_{14} to C_{18}) fatty acyl-CoA synthetase genes, *FAA1* and *FAA4*, blocks incorporation of long-chain saturated fatty acids and UFAs such as oleic acid into cells and does not cause UFA repression of *OLE1* transcription (9, 10). A putative fatty acid transport protein (FATP) with 33% homology to an adipocyte FATP (42) was identified (15). However, more-recent reports indicate that this protein is an acyl-CoA synthetase specific for very-long-chain fatty acids rather than a plasma membrane fatty acid transporter (9, 52). Therefore, a fatty acid transport protein and UFA signal transducers and responding transcriptional regulators have not yet been identified.

Since the desaturation reaction requires molecular oxygen (O_2) as an electron acceptor (35), it has been proposed that cells derepress expression of *OLE1* under hypoxic conditions to allow more-efficient use of limiting O_2 (53). Well-established O_2-responsive repressor Rox1p is believed to repress *OLE1* transcription under aerobic conditions because putative Rox1p-binding sequences are present in the *OLE1* promoter (53). However, there had been no conclusive evidence that *OLE1* transcription is indeed repressed by O_2 until the recent report by Kwast et al. (26), who showed that *OLE1* transcription was induced when cells were shifted to anaerobic conditions.

In this report, we show that O_2 repression of *OLE1* is mediated by a Rox1p-independent novel mechanism. Furthermore, we newly identified a 50-bp region essential for O_2 repression, designated the O2R element, and this element was also responsible for UFA repression of anaerobically derepressed *OLE1* transcription despite not being included within the FAR element. Therefore, there appears to be a close connection between O_2 and UFA signals that act on the short 50-bp element in the *OLE1* promoter.

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TABLE 1. *S. cerevisiae* strains used

Strain	Genotype[a]	Source
SH4041	*MAT***a** *ura3 trp1 leu2-3,112 his3*Δ *pho3-1 pho5-1*	Our laboratory
SH5128	*MAT***a** *ura3 trp1 leu2-3,112 his3*Δ *pho3-1 pho5-1 rox1::LEU2*	This study
SH5420	*MAT***a** *ura3*::[*URA3 OLE1*p-*PHO5*] *trp1 leu2-3,112 his3*Δ *pho3-1 pho5-1*	This study
SH5421	*MAT***a** *ura3*::[*URA3 OLE1*p-*PHO5*] *trp1 leu2-3,112 his3*Δ *pho3-1 pho5-1 rox1::LEU2*	This study
SH5143	*MAT*α *ura3-52 leu2-3,112 his4-519 trp1 pho3-1 pho5-1 can*^{r}	Our laboratory
SH5141	*MAT*α *ura3-52*::[*URA3 OLE1*p-*PHO5*] *trp1 leu2-3,112 his4-519 pho3-1 pho5-1 can*^{r}	This study

[a] *ura3*::[*URA3 OLE1*p-*PHO5*] and *ura3-52*::[*URA3 OLE1*p-*PHO5*] represent the integration of the *OLE1*p-*PHO5* fusion reporter gene into the *ura3* locus using the plasmid p1166 as described in Materials and Methods. Other symbols are as described previously (32).

MATERIALS AND METHODS

Microorganisms and media. The *S. cerevisiae* strains used in this work are listed in Table 1. *Escherichia coli* strains TG1 (40) and DH5α (40) were used as hosts for the propagation and manipulation of plasmid DNA. Yeast cells were grown in nutrient medium (YPDA; i.e., nutrient high-P_i medium) (38) or YPDA supplemented with 1 mM oleic acid (Wako Chemicals, Osaka, Japan) and 1% (vol/vol) Triton X-100 (Wako Chemicals) (16). The Luria-Bertani medium for *E. coli* was as described previously (40).

Disruption of *ROX1*. A 1,109-bp fragment of *ROX1* (nucleotides [nt] −2 to +1107) was amplified by PCR using chromosomal DNA of *S. cerevisiae* strain S288C (33) as a template and oligonucleotides 5′-GCGGATCCCAATGAATC CTAAATCCTCTACAC-3′ and 5′-CGCTCGAGTCATTTCGGAGAAACTA GGC-3′, corresponding to the nt −2 to +22 and +1107 to +1088 of *ROX1*, as forward and reverse primers, respectively. The PCR product was doubly digested with *Bam*HI and *Xho*I and cloned into the *Bam*HI-*Xho*I gap of pRS305 (44) to obtain plasmid p1513. A 1,148-bp fragment of *ROX1* (nt −1121 to +27) was amplified by PCR using chromosomal DNA of S288C as a template and oligonucleotides 5′-CCAAGCTTCCATTGAGAAGGACAACATT-3′ and 5′-CTGG ATCCTTAGGTGTAGAGGATTTAGG-3′, corresponding to the nt −1121 to −1102 and +27 to +7 of *ROX1*, as forward and reverse primers, respectively. The PCR product was doubly digested with *Hin*dIII and *Bam*HI and cloned into the *Hin*dIII-*Bam*HI gap of pUC18 (40) to obtain plasmid p1514. A 2.9-kb *Bam*HI-*Sca*I fragment from p1513 and a 2.1-kb *Bam*HI-*Sca*I fragment from p1514 were ligated to obtain plasmid p1515. A 525-bp *Bam*HI-*Bgl*II fragment of p1515 containing the *ROX1* open reading frame (ORF), corresponding to nt +28 to +523, was replaced with a 1.7-kb *Bam*HI fragment containing *LEU2* from YDp-L (4) to obtain plasmid p1516. The *rox1::LEU2* disruptant, SH5128, was constructed by transformation of SH4041 with *Hin*dIII- and *Xho*I-digested plasmid p1516. The *rox1::LEU2* disruption was verified by Southern analysis.

***OLE1*p-*PHO5* fusions.** Construction of plasmid p1166, which contains a 935-bp fragment upstream of *OLE1* fused with the structural region of *PHO5* encoding repressible acid phosphatase (rAPase; EC 3.1.3.2), was described previously (16). The activation and repression assay vector pRAV, containing the UAS_{PHO84}E-*PHO84*p-*PHO5* reporter gene, was constructed previously (31). The UAS_{PHO84}E-*PHO84*p-*PHO5* reporter gene consists of a 272-bp fragment upstream of *PHO84* containing Pho4p binding site E fused with the *PHO5* ORF. Plasmid p1785 (see Fig. 2), which has the −934 to −586 region of *OLE1* (taking base A of the ATG start codon as +1) upstream of the UAS_{PHO84}E-*PHO84*p-*PHO5* reporter gene was constructed as follows. A 349-bp region of *OLE1* was amplified by PCR using p1166 as a template and oligonucleotides 5′-CTCGAA TTCAGCTTTTCGTTTGCAGGTTT-3′ and 5′-CTCAAGCTTAGTTAGTTTT TGGGCCACCG-3′, corresponding to the nt −934 to −915 and −586 to −605 of *OLE1*, as the forward and reverse primers, respectively. The PCR product was doubly digested with *Eco*RI and *Hin*dIII and cloned into the *Eco*RI-*Hin*dIII gap of pRAV to obtain plasmid p1785. Plasmids p1787, p1781, p1783, p1860, p1862, and p1864, which each contain nt −585 to −456 (the FAR element), −455 to −307, −306 to −159, −455 to −406, −405 to −357, and −356 to −307 of *OLE1*, respectively, were constructed in a similar way to plasmid p1785 by inserting *Eco*RI-*Hin*dIII fragments containing each region amplified by PCR into the *Eco*RI-*Hin*dIII gap of pRAV. Plasmid p1872, which carries *OLE1*p lacking the O2R element (*OLE1*pΔO2R) fused with the structural region of *PHO5* was constructed as follows. The −306 to −1 region of *OLE1* was amplified by PCR using p1166 as a template and oligonucleotides 5′-CTCAAGCTTTTCTACGA GTCTTGCTCACT-3′ and 5′-GCGGATCCTTTGTTGTAATGTTTTAG-3′, corresponding to the nt −306 to −287 and −1 to −19 of *OLE1*, as the forward and reverse primers, respectively. The PCR product was doubly digested with *Hin*dIII and *Bam*HI and cloned into the *Hin*dIII-*Bam*HI gap of pSH39 (34) to obtain plasmid p1870. The −934 to −357 region of *OLE1* was amplified by PCR using p1166 as a template and oligonucleotides 5′-CTCGAATTCAGCTTTTC GTTTGCAGGTTT-3′ and 5′-CTCAAGCTTAAAGAAAGCTGCCGACTAT G-3′, corresponding to nt −934 to −915 and −357 to −376 of *OLE1*, as the forward and reverse primers, respectively. The PCR product was doubly digested with *Eco*RI and *Hin*dIII and cloned into the *Eco*RI-*Hin*dIII gap of p1870 to obtain plasmid p1872. Plasmid p1904, in which the 5′-GATAA-3′ sequence in the O2R element is changed to 5′-ACGCC-3′ by site-directed mutagenesis, was constructed as follows. Oligonucleotides 5′-AATTCCGGACGTTGAAACACT CAACAAACCGGCGTTAGTGCCCAACCAGGTGTGCA-3′ and 5′-AGCTT GCACACCTGGTTGGGCACTAACGCCGGTTTGTTGAGTGTTTCAACG TCCGG-3′, corresponding to nt −356 to −307 and −307 to −356 of *OLE1*, respectively, in which the 5′-GATAA-3′ sequence (−331 to −327) is changed to 5′-ACGCC-3′ (underlined) were annealed and cloned into the *Eco*RI-*Hin*dIII gap of pRAV to obtain plasmid p1904. Plasmid p1858, which contains a 567-bp fragment upstream of *ANB1* (27) fused with the *PHO5* ORF, was constructed as follows. A 567-bp fragment of *ANB1* (nt −567 to −1) was amplified by PCR using chromosomal DNA of S288C as a template and oligonucleotides 5′-CTC AAGCTTCCGGGAATTTTAGATTCAGG-3′ and 5′-CTCGGATCCGTTTTA GTGTGTGAATGAAA-3′, corresponding to nt −567 to −548 and −1 to −20 of *ANB1*, as the forward and reverse primers, respectively. The PCR product was doubly digested with *Hin*dIII and *Bam*HI and cloned into the *Hin*dIII-*Bam*HI gap of pSH39 to obtain plasmid p1858. All constructs were analyzed by sequencing the respective promoter regions. The resulting plasmids were digested with *Stu*I and integrated into the *URA3* locus of SH5143, SH4041, or SH5128 by transformation. Single-copy integration was confirmed by Southern analysis of genomic DNA digested with *Hin*dIII from the respective transformants.

Northern blot analysis. The preparation of RNA and Northern blot hybridization were performed as described previously (38). Cells were cultivated in 10 ml of YPDA medium to stationary phase at 30°C with vigorous shaking. The stationary-phase culture was inoculated into 30 ml of YPDA or YPDA containing oleic acid in a 100-ml Erlenmeyer flask to give an optical density at 660 nm (OD_{660}) of 0.1. The cultures were shaken at 30°C for aerobic growth. For anaerobic growth, the cultures were sealed with rubber stoppers and bubbled with pure nitrogen gas for 2 min to purge O_2 after inoculation or sampling, and shaken at 30°C. Anaerobic conditions were verified based on confirming the transcription of *ANB1* (28), a well-known hypoxic gene. Total RNAs were prepared from the cells harvested in the logarithmic growth phase (OD_{660} = 0.7 to 1.6). DNA fragments containing the *OLE1* ORF (nt −9 to +1905 relative to ATG), the *PHO5* ORF (nt −18 to +2116), and the *ANB1* ORF (nt +1 to +455), amplified by PCR, and the 1.0-kb *Hin*dIII-*Xho*I fragment carrying *ACT1* prepared from pYA301 (19) were labeled with ^{32}P as described previously (39) to generate DNA probes.

rAPase assay. Cells were cultivated in 10 ml of YPDA medium to stationary phase at 30°C with vigorous shaking. The stationary-phase culture (0.2 ml) was inoculated into 10 ml of YPDA medium or YPDA medium containing oleic acid. The cultures were shaken for 5 h at 30°C for aerobic growth or left to stand for 24 h at 30°C in a sealed Anaero Pack (Mitsubishi Gas Chemical Co., Tokyo, Japan) for anaerobic growth, and then rAPase activities were measured as described previously (48). The rAPase activities presented are the averages of at least three independent experiments.

Genetic and biochemical methods. *S. cerevisiae* and *E. coli* cells were transformed as described by Ito et al. (24) and Sambrook et al. (40), respectively. Yeast chromosomal DNA was prepared as described previously (23). Southern blot analysis and other DNA manipulations were performed using standard methods (40). Bacterial plasmid DNA was isolated by the alkaline lysis method (40). Nucleotide sequences were determined by the dideoxy chain termination method (41).

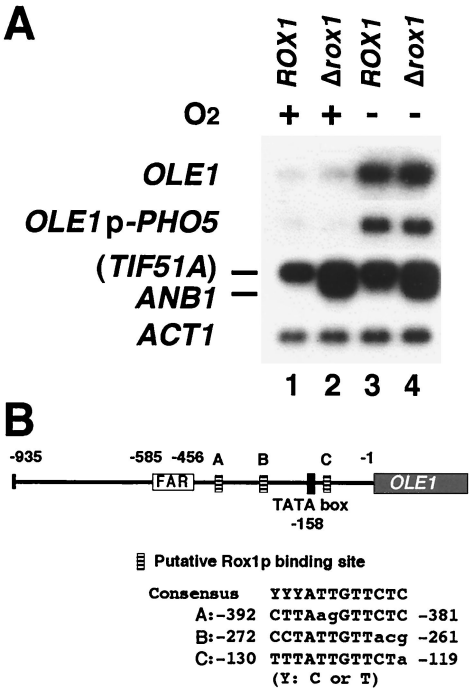

FIG. 1. Transcription of *OLE1* is repressed by O_2 in a Rox1p-independent manner. (A) Northern analysis of the *OLE1*, *OLE1*p-*PHO5*, and *ANB1* transcripts in *rox1* disruptants. Total RNA samples were prepared from cells of the wild-type strain (SH5420) and *rox1* disruptant (SH5421) cultivated aerobically (lanes 1 and 2) or anaerobically (lanes 3 and 4) in YPDA medium. Equal amounts of RNA (5 μg) were electrophoresed in a 1.5% agarose gel in the presence of formaldehyde, transferred to a nylon filter, blotted, and hybridized with probes consisting of ^{32}P-labeled DNA fragments containing *OLE1*, *ANB1*, and *PHO5* for detection of the transcripts of *OLE1*p-*PHO5* or *ACT1* as an internal control. The *ANB1* probe also hybridized to the *TIF51A* transcript, which is not repressed by Rox1p (53). (B) Putative Rox1p binding sites in the *OLE1* promoter. Numbers indicate positions relative to the first nucleotide of the initiation codon (+1). Lowercase letters, nucleotides that differ from the consensus Rox1p-binding sequence (3).

RESULTS AND DISCUSSION

Transcription of *OLE1* is repressed by O_2 in a Rox1p-independent manner. To clarify whether *OLE1* transcription is indeed repressed by O_2, we investigated *OLE1* transcription in cells cultivated aerobically or anaerobically. Northern blot analysis (Fig. 1A) showed that the levels of transcripts of *OLE1* and *OLE1*p-*PHO5* fusion gene are significantly increased under anaerobic conditions, compared to the levels under aerobic conditions, in the wild-type strain (Fig. 1A, lanes 1 and 3), indicating that *OLE1* transcription is repressed by O_2. The same observation that *OLE1* transcription is induced when cells are shifted to anaerobic conditions has been reported recently (26). In *S. cerevisiae*, a Rox1p-dependent mechanism is known to mediate transcriptional repression of various hypoxic genes by O_2 (53). Since the biosynthesis of heme requires O_2, heme accumulates under aerobic conditions and binds to Hap1p. Hap1p with bound heme acts as a transcriptional activator to activate *ROX1* transcription. Rox1p binds to its recognition sites, 5′-YYYATTGTTCTC-3′ (where Y represents pyrimidine) (3), upstream of anaerobically expressed genes such as *ANB1* and forms a complex with the general repressors Tup1p and Ssn6p to repress target genes under aerobic conditions (53). As three putative binding sites for Rox1p are present in the *OLE1* promoter (Fig. 1B), we disrupted *ROX1* to determine whether *OLE1* repression requires Rox1p. In the *rox1* disruptant, *ANB1* transcription was, as reported previously (53), derepressed under aerobic conditions (Fig. 1A, lane 2). However, *OLE1* transcription was unexpectedly not derepressed (Fig. 1A, lane 2), indicating that Rox1p is not involved in the O_2 repression of *OLE1*. Although there are many hypoxic genes which have consensus binding sequences for Rox1p in their promoters (53), for some of them the function of these sequences has not been experimentally determined by, for example, deletion or mutation analysis of the consensus sequence or disruption of *ROX1*. Our results for *OLE1* demonstrate that one must not decide on the operating mechanism only by the presence of a consensus sequence. What is the mechanism of O_2 repression of *OLE1*? Recently, another group has shown that the respiratory chain is involved in the anaerobic induction of *OLE1* transcription and that cytochrome *c* oxidase is likely the hemoprotein sensor for O_2 (26). However, nothing is known about the signal transduction pathway downstream of the O_2 sensor. Identification of O_2-responsive transcriptional factors involved in Rox1p-independent O_2 repression should make it possible to elucidate the repression mechanism.

The 50-bp O2R element is sufficient and essential for O_2 repression of *OLE1* transcription. To elucidate the Rox1p-independent O_2 repression mechanism of *OLE1*, we first identified the O_2 signal-responsive region in the *OLE1* promoter. The *OLE1* promoter was divided into three subregions, i.e., the region harboring the FAR element and the regions upstream and downstream of this region, and these regions were inserted into the region upstream of the *PHO84*p-*PHO5* reporter gene in the activation and repression assay vector pRAV (31). The transcription of the *PHO84*p-*PHO5* reporter gene is repressed when cells are cultivated under conditions whereby a sufficient amount of P_i is used, such as YPDA medium (31). The levels of expression of these reporter genes were measured as rAPase activities under aerobic and anaerobic conditions (Fig. 2). Control experiments with the *ANB1*p-*PHO5* reporter gene (p1858) validated this reporter assay system to monitor O_2-mediated transcriptional repression. The rAPase activity from p1166 was derepressed 4.4-fold under anaerobic conditions compared with aerobic conditions. Although p1787, which contains a FAR element, was not as responsive to anaerobic conditions (rAPase activity was derepressed 1.4-fold), p1781, containing the downstream region of the FAR element, responded significantly (rAPase activity was derepressed 9.7-fold). In order to delineate the O_2-regulated element in the 149-bp *OLE1*p region of p1781, we further divided this region into three subregions and determined which region responds to anaerobic conditions. Only p1864 showed significant derepression of rAPase activity (7.3-fold) under anaerobic conditions, indicating that the 50-bp *OLE1*p region (nt −356 to −307) is sufficient for anaerobic derepression. Deletion of the 50-bp region from *OLE1*p (p1872) decreased the level of anaerobic derepression from 4.4- to 1.4-fold, indicating that the 50-bp region is essential for anaerobic derepression of *OLE1* transcription. We therefore designated the 50-bp region as an O_2-regulated element (O2R). That the O2R element does not contain consen-

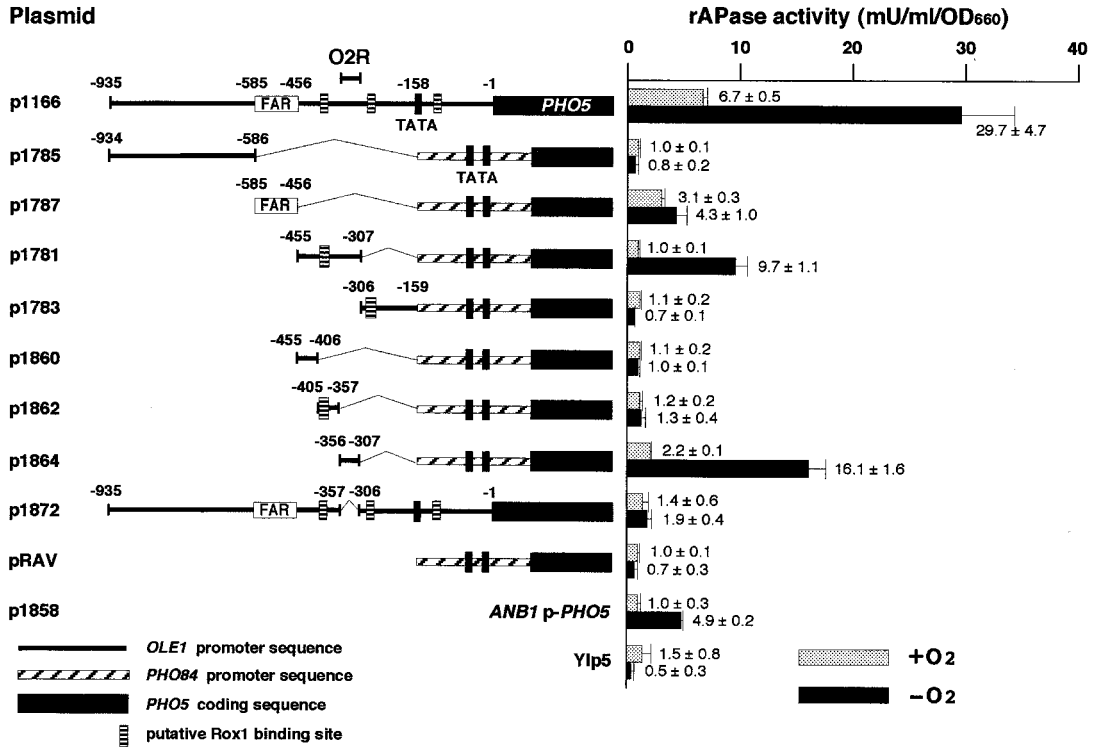

FIG. 2. The O2R element is present in the −356 to −307 region of the *OLE1* promoter. rAPase activity in cells of the wild-type strain (SH5143) harboring the respective reporter genes cultivated aerobically ($+O_2$) or anaerobically ($-O_2$) in YPDA medium was measured as described previously (48). Numbers indicate the position relative to the first nucleotide of the initiation codon (+1) of *OLE1*. *PHO84* promoter sequences (nt −272 to −1) contain binding site E (nt −262 to −257) for the transactivator Pho4p and two putative TATA boxes (nt −122 and −99). Shaded boxes, structural regions of *PHO5*. The *ANB1*p-*PHO5* reporter gene was used to verify anaerobic conditions. Error bars, standard deviations determined from a minimum of three independent measurements. The actual values of rAPase activity with their standard deviations are indicated. Other symbols are the same as those described for Fig. 1B.

sus Rox1p-binding sites is consistent with a Rox1p-independent mechanism for O_2 repression of *OLE1* transcription (Fig. 2). These results indicate that the O2R element is an anaerobic upstream activation site and further imply that some anaerobically responsive activators acting on the element exist. Thus, the system for O_2 repression of *OLE1* transcription appears to be similar to the mammalian HIF-1 hypoxia-sensing system, whose regulation is mediated by transcriptional activator HIF-1 (51). On the other hand, the Rox1p-dependent O_2 repression system is not parallel to the HIF-1 hypoxia-sensing system because it is mediated by transcriptional repressor Rox1p, which binds to the upstream repression site of O_2-repressed genes (53). It is noted that a homologue of HIF-1 is not found in *S. cerevisiae* (21) and that the O2R element does not contain the consensus core sequence, 5′-RCGTG-3′, of HIF-1 binding sites (43).

5′-GATAA-3′ sequence in the O2R element is important for O_2 repression. What is the transcriptional activator acting on the O2R element? We searched for binding motifs of already-known transcriptional regulators on the O2R element and found the 5′-GATAA-3′ sequence, which is the binding site for GATA family transcriptional activation factors Gln3p and probably Gat1p (Fig. 3A) (5, 13). Although the 5′-GATAA-3′ sequence is on the complementary strand of the O2R element, it is known that the 5′-GATAA-3′ sequence functions in an orientation-independent manner (12). The GATA family of DNA-binding proteins are present in organisms from *S. cerevisiae* to humans (29, 30). These binding proteins contain one or more characteristic C_4 zinc finger motifs and bind to DNA sequences with sequence GATA at their cores (11). In vertebrates, six GATA factors, GATA-1, GATA-2, GATA-3,

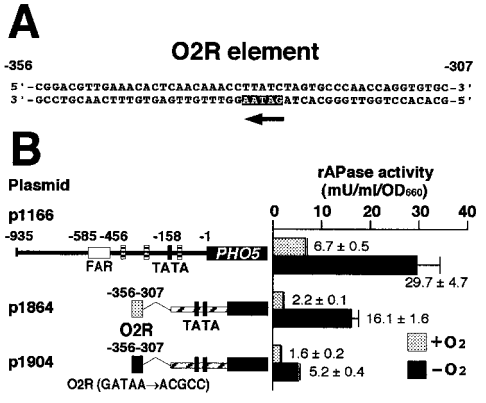

FIG. 3. (A) The O2R element contains a 5′-GATAA-3′ sequence (arrow) on the complementary strand. (B) The 5′-GATAA-3′ sequence plays an important role in O_2 repression. Dotted box, O2R element (nt −356 to −307); solid box, O2R in which the 5′-GATAA-3′ sequence is changed to 5′-ACGCC-3′ by site-directed mutagenesis. The conditions used for measurement of rAPase activity in cells of the wild-type strain (SH5143) harboring the respective reporter genes and the symbols employed are as described in the legend to Fig. 2.

GATA-GT1, GATA-GT2, and GATA-5, have been identified and are involved in regulation of various genes such as those encoding globins, erythropoietin receptors, preproendothelin-1, T-cell receptors, and the gastric proton pump (29). To determine whether the 5′-GATAA-3′ sequence on the O2R element functions in O_2 repression, we mutated the 5′-GA TAA-3′ sequence to 5′-ACGCC-3′. As shown in Fig. 3B, this mutation resulted in a decrease in the level of anaerobic derepression from 7.3- to 3.3-fold, indicating that the 5′-GAT AA-3′ sequence is important in O_2 repression. Since it is known that there are only four GATA factors, i.e., transcriptional activators Gln3p and Gat1p and transcriptional repressors Dal80p and Deh1p, in *S. cerevisiae* (11), Gln3p and Gat1p could be candidates for the transcriptional activator acting on the O2R element. These four proteins respond to the nitrogen source signal to regulate nitrogen catabolite-repressed genes (11) but have not been reported to be involved in transcriptional regulation by O_2 or UFAs. However, in vertebrates, GATA-1 has been demonstrated to play a major role in the regulation of various specific erythroid genes, for example, genes encoding globins and heme biosynthetic enzymes involved in terminal erythroid differentiation in vivo (14, 36, 37, 47, 49, 50). This finding appears to imply a relationship between GATA factors and O_2 signals.

In *S. cerevisiae*, transcription of *ATF1*, encoding alcohol acetyltransferase, is also repressed by O_2 and UFA (18). Fujiwara et al. identified a 51-bp region (nt −150 to −100) responsible for O_2 repression and an 18-bp region (nt −85 to −68) responsible for UFA repression of *ATF1* (17). The O_2-responsive 51-bp region contains a Rox1p binding site, and O_2 repression was partially abolished in the *rox1* null mutant (17). There is no homology between the O2R element and the 51-bp region of *ATF1*, and the 51-bp region does not contain the 5′-GATAA-3′ sequence. From these facts, we conclude that the O_2 repression mechanism of *OLE1* transcription is different from that of *ATF1* transcription.

O_2 and UFA signals act on the O2R element. To further clarify the relationship between O_2 and UFA repression mechanisms, we investigated the effect of UFAs on anaerobic derepression of *OLE1* transcription. Northern blot analysis showed that anaerobic derepression of *OLE1* transcription (Fig. 4A, lanes 1 and 2) did not occur in the presence of UFA (Fig. 4A, lanes 3 and 4). This suggests that UFA repression is epistatic to anaerobic derepression. From a physiological viewpoint, this result is reasonable because, if there is a sufficient amount of UFA in the medium, cells do not have to produce UFA even under anaerobic conditions.

However, these results are different from those of Kwast et al. (26). They reported that *OLE1* transcription is derepressed when cells are shifted to anaerobic conditions even in the presence of UFAs. We think that this difference may be due to the source or concentration of UFA because we used free oleic acid at a concentration of 1 mM while they used Tween 80, which is an oleic acid ester, at a concentration of 0.1% (vol/vol) (approximately 0.6 mM). Our experimental conditions might be more highly repressive than theirs. If an excess amount of UFA is incorporated by cells under their experimental conditions, the anaerobic derepression may not occur, as was the case in our study.

As UFA repression of *OLE1* transcription depends on the

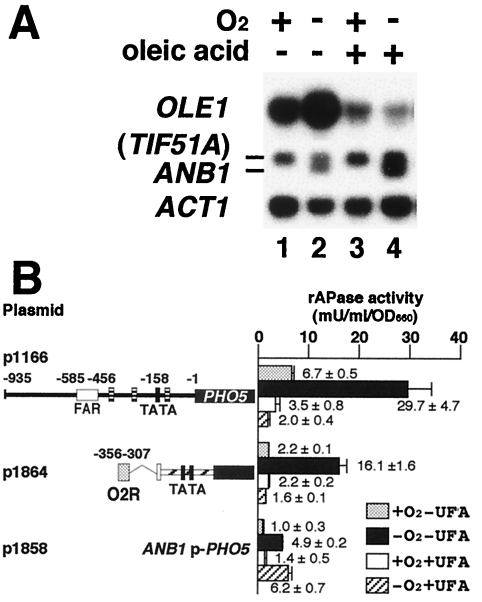

FIG. 4. (A) Anaerobic derepression of *OLE1* transcription is repressed by oleic acid. Cells of the wild-type strain (SH5141) were inoculated into YPDA medium (lanes 1 and 2) or YPDA medium containing 1 mM oleic acid (lanes 3 and 4) and cultivated at 30°C aerobically (lanes 1 and 3) or anaerobically (lanes 2 and 4). Total RNA was prepared from cells in the logarithmic growth phase. The RNA samples (each 10 μg of RNA) were subjected to Northern blot hybridization as described in the legend to Fig. 1A. (B) The O2R element is responsible for repression by UFA. Cells of SH5143 (wild type) harboring one copy of the respective reporter genes integrated at the *ura3* locus were cultivated aerobically without oleic acid (dotted bars), anaerobically without oleic acid (solid bars), aerobically with oleic acid (open bars), or anaerobically with oleic acid (hatched bars) and subjected to an rAPase assay as described in the legend to Fig. 2.

FAR element under aerobic conditions, the FAR element has been considered to be the sole element which responds to UFA signals in the *OLE1* promoter (10). To determine whether UFA repression of *OLE1* transcription under anaerobic conditions also depends on the FAR element, we examined the effect of UFAs on the O2R element only. Unexpectedly, despite not being included within the FAR element, the O2R element was responsible for this UFA repression (Fig. 4B). The fact that the short 50-bp O2R element responds to both O_2 and UFA signals implies that the signal transduction pathways of O_2 and UFA merge in the ultimate step of the pathways.

Our data together with those of other researchers (10) suggest that the FAR element plays a major role in the production of UFAs under aerobic conditions (Fig. 2; p1787), while the O2R element plays a major role under anaerobic conditions (Fig. 2; p1864) because it has a higher potential for transcriptional activation than the FAR element. Based on these findings we propose a model for *OLE1* transcriptional regulation by O_2 and UFA signals (Fig. 5). According to our model, under aerobic conditions in the absence of UFAs, *OLE1* transcription is derepressed mainly by unknown transcriptional activators acting on the FAR element. Under anaerobic conditions in the absence of UFAs, a different transcriptional activator which acts on the newly identified O2R element plays a major role in further derepression of *OLE1* transcription for

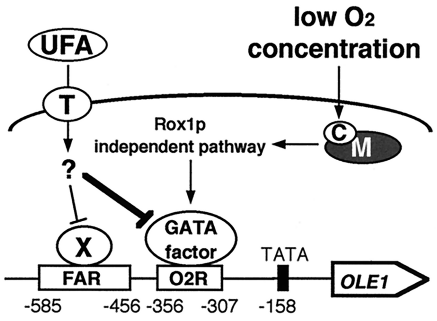

FIG. 5. A model for signal transduction pathways of O_2 and UFA regulating *OLE1* transcription. X, unknown transcriptional activators acting on the FAR element; M and C, mitochondrial respiratory chain and cytochrome *c* oxidase, respectively; T, unknown fatty acid transporter located at the cellular membrane. Arrows and blunt arrows, positive and negative interactions, respectively. UFA repression is epistatic to anaerobic derepression (thick line). See Discussion for details.

the efficient use of limiting O_2 by cells. The GATA factors Gln3p and Gat1p are candidates for the unknown activator. The anaerobic signal is transmitted in a Rox1p-independent manner. The mitochondrial respiratory chain is involved in the anaerobic induction of *OLE1* transcription, and cytochrome *c* oxidase is likely the hemoprotein sensor for O_2 (26). On the other hand, under aerobic conditions in the presence of UFAs, the UFAs are transported into cells by an unidentified transporter located at the cellular membrane and repress *OLE1* transcription by inhibiting activators acting on the FAR element. Under anaerobic conditions in the presence of UFAs, the UFAs repress *OLE1* transcription by inhibiting both FAR- and O2R-dependent transcriptional activators.

In *S. cerevisiae*, the hypoxic genes fall into at least two classes (53). One class comprises single-copy genes and includes *OLE1*, *ERG11*, *CPR1*, *HEM13*, and *SUT1*. These genes encode enzymes expressed at low levels under aerobic conditions, but expression levels increase as oxygen becomes limiting. The second class represents gene pairs, where one gene is expressed under aerobic conditions while the other gene is expressed under hypoxic conditions. These gene pairs include *COX5a*/*COX5b*, *CYC1*/*CYC7*, *AAC2*/*AAC3*, and *TIF51a*/*ANB1* (25, 53). The products of gene pairs *COX5a*/*COX5b* and *CYC1*/*CYC7* have been shown to influence the maximal turnover number of holocytochrome *c* oxidase, with the hypoxic isoforms increasing this rate (2, 7). On the basis of these facts, we argue that cells regulate the expression of single-copy hypoxic genes by switching on regulatory elements of the genes, such as the FAR and O2R elements in the case of *OLE1*, instead of switching on the expression of the gene pairs responding to environmental O_2 signals.

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