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Genetics

Oxygen regulation of anaerobic and aerobic genes mediated by a common factor in yeast

(coordinate expression/gene regulation/gene fusion/transcriptional regulation)

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ABSTRACT **The expression of a number of yeast genes is regulated by oxygen levels. While many of these are known to be induced in the presence of oxygen, we have described a gene, *ANB1*, that responds in the opposite fashion, being expressed only under anaerobic conditions. To identify genes involved in regulation of *ANB1* and other oxygen-regulated genes, we selected mutations causing constitutive expression of *ANB1*, using a fusion of the *ANB1* modulator segment to the *CYC1* gene. A number of *trans*-acting mutations affecting a gene designated *ROX1* caused constitutive expression of both the fused and wild-type genes, indicating that the *ROX1* gene product operates through the *ANB1* modulator sequence at the level of transcription. The mutant alleles of *ROX1* fall into two phenotypic classes. The *rox1-a* class is semi-dominant, and the *rox1-b* class is recessive. One mutant, *rox1-a1*, is pleiotropic and causes constitutive expression of three oxygen-induced genes—*CYC1*, *SOD* (superoxide dismutase), and *tr-1* (an oxygen-induced gene with homology to *ANB1*)—as well as constitutive expression of the oxygen-repressed *ANB1* gene. Alleles of the *rox1-b* class cause constitutive expression of *ANB1* but do not affect expression of the oxygen-induced genes tested. The pleiotropy of the *rox1-a1* mutant indicates that the *ROX1* gene product is involved in coordinate expression of both oxygen-induced and oxygen-repressed genes.**

Many eukaryotic genes can be grouped into families defined by their coordinate expression in response to extracellular conditions or signals (1). These gene families are usually dispersed throughout the genome. For the expression of a dispersed gene family to be regulated by a common mechanism—a feature seemingly desirable for economy and efficient coordination—it has been suggested that common factors, specific for each gene family, form regulatory complexes at common recognition sequences within the transcriptional modulators lying upstream from each gene (1). The role of such consensus sequences (1–4) and of common regulatory factors (5–14) has been indicated in several systems.

We have sought to determine whether a group of genes in *Saccharomyces cerevisiae* whose expression is regulated by the presence of oxygen are controlled by common factors acting through common regulatory sites. The expression of one such gene, *CYC1*, has been investigated in detail, as reported from this and other laboratories. Regulation of expression by oxygen levels has been shown to be mediated at an upstream transcriptional modulator site (15, 16), with the involvement of heme as a regulatory effector (16, 17). Expression of other oxygen-induced genes is also dependent on heme (17, 18), regulated by it at the level of transcription (17). These findings are consistent with the hypothesis of coordinate expression mediated by a common mechanism. In addition, pleiotropic mutations affecting oxygen regulation of some genes coding for hemoproteins have been reported (17).

We have also studied another gene whose expression is regulated by oxygen, but in the opposite way. The anaerobic gene *ANB1* (previously designated *tr-2*), whose function is unknown, is expressed only in the absence of oxygen (15). We reasoned that, since both the aerobically and anaerobically controlled genes are regulated by oxygen, common factors might be involved in their expression, so that *trans*-acting mutations selected for an effect on one gene might affect the others. To test this, we selected for constitutive mutations causing accumulation of *ANB1* mRNA in the presence of oxygen. The mutants obtained define a regulatory gene, which we designate *ROX1*. In agreement with the hypothesized involvement of common regulatory factors in coordinate expression, one of the mutant alleles of *ROX1* proved to be pleiotropic, causing oxygen-constitutive expression of aerobic genes as well as of *ANB1*. This provides an example of coordinated regulation of two sets of genes whose expression responds in an opposite way to the same environmental factor.

MATERIALS AND METHODS

Strains. The parental strain was GM-3C-2 [*MATα*, *cyc1-1*, *cyp3-1* (*cyc7*), *trp1-1*, *his4-519*, *leu2-3*, *leu2-112*, *Gal*$^-$; see ref. 19]. For genetic analysis, crosses were made with aRZ5 (*MATa*, *cyc1-1*, *cyp3-1*, *ura3*, *his4-519*). Some genetic tests were performed with strains CL-2-15a (*MATα*, *rox1-a1*, *cyc1-1*, *cyp3-1*, *trp1-1*, *ura3*, *his4-519*) and CL1-1-13b (*MATa*, *rox1-b3*, *cyc1-1*, *cyp3-1*, *trp1-1*, *leu2-2*, *leu2-112*, *his4-519*).

Plasmids and Transformation. The following plasmids were used for transformation of GM-3C-2: YCp*CYC1*(2.4) (see Fig. 1; ref. 15); YCp*CYC1*(2.4)1/X (see Fig. 1; ref. 15); YEp13 (20), which carries the *LEU2* gene; and YEp*HO* [supplied to us by R. Jensen and I. Herskowitz (21)], which carries the *HO* mating-type switching gene cloned in YEp13. Plasmids were introduced into yeast or *Escherichia coli* strain HB101 by transformation as described (15). Strains were cured of centromeric plasmids by growing cells for 20 generations in YPD medium (2% peptone/1% yeast extract/3% glucose), spreading them on YPD plates, and replica-plating on CM (*–trp*) plates to identify colonies that had lost the plasmid.

Plasmid pFL1-cIII-FeS-3 (ref. 22; supplied to us by L. Grivell) was recently shown to carry the superoxide dismutase gene (*SOD*) (L. Grivell, personal communication) on a 1.8-kilobase (kb) *Bam*HI fragment, which was used as a hybridization probe for *SOD* mRNA. Other probes were derived from YCp*CYC1*(2.4) or pYe*CYC1*(0.6) (23) as indicated in the text.

Mutagenesis and Selection of Constitutive Mutations. GM-3C-2 cells transformed with YCp*CYC1*(2.4)1/X were mutagenized with ethyl methanesulfonate or UV irradiation, as

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Abbreviation: kb, kilobase(s).

described by Sherman *et al.* (24). After mutagenesis, cells were allowed to grow for ≈6 generations in YPD before plating. Mutant colonies were selected for growth on CM (lactate) plates.

Media and Growth Conditions. Synthetic media [CM (glucose) and CM (lactate)] were as described (15). For RNA analysis, aerobic cultures were shaken at 30°C at 250 rpm in large flasks (10 times the culture volume) in YPD or in YPR medium (2% peptone/1% yeast extract/2% raffinose). Cells were harvested at 10^7 cells per ml after swirling for 5 min on ice. Anaerobic cultures inoculated with a fresh stationary phase culture were grown in YPD from an initial density of 10^6 cells per ml to a final density of 10^7 cells per ml. After 15 min of vigorous bubbling with ultrapure (99.998%; Linde) nitrogen, the cultures were shaken at moderate speed with slow bubbling for 7–8 hr. Before opening, the flasks were chilled for 15 min by addition of crushed ice to the shaking water bath. Cells were harvested at 0°C.

Extraction and Analysis of RNA. RNA was extracted from yeast cells and analyzed by gel blot as described (15) onto Gene-Screen (New England Nuclear) membranes. When necessary, these blots were stripped of probe as described by Thomas (25) and rehybridized after the usual period of prehybridization. In some cases, this was repeated several times with a series of different probes.

Genetic Analysis. For complementation analysis, mutants were cured of YCp*CYC1*(2.4)1/X and converted from *MATα* to *MATa* by transformation with YEp*HO* (21). The parental strain, GM-3C-2, is defective in switching from *MATa* to *MATα*, so that YEp*HO* transformants were conveniently recovered in homogeneous *MATa* colonies. After curing of YEp*HO* and retransforming with YEp13 (*LEU2*), the *MATa* mutants were mated with appropriate *MATα* mutants carrying YCp*CYC1*(2.4)1/X (*TRP1*), and isogenic diploids (*LEU2*, *TRP1*) were selected. Pairs of mutations were judged to complement if diploids failed to grow on CM (lactate) plates. Because of negligible sporulation by these diploids, tetrad analysis was done on diploids resulting from crosses between aRZ5 and mutants carrying YCp*CYC1*(2.4). These cells sporulated at a frequency of 20%–30% when induced to do so by standard methods (24).

RESULTS

Cells of the respiratory-deficient strain GM-3C-2 (*cyc1*, *cyc7*) are unable to grow on nonfermentable media. This deficiency can be complemented by transformation with a chimeric plasmid YCp*CYC1*(2.4) (15), which carries the *CYC1* gene on a 2.4-kb fragment. This fragment also contains a neighboring gene, *ANB1*, which is completely repressed during aerobic growth and induced during anaerobic growth. In earlier work, a mutation had been constructed in the YCp*CYC1*(2.4) plasmid in which a control region lying between the two genes was inverted, placing *CYC1* transcription under the control of the transcriptional modulator of the *ANB1* gene, forming a regulatory fusion (*ANB1/CYC1*) between the two genes (Fig. 1; ref. 15). In this configuration, *CYC1* mRNA is produced only in the absence of oxygen, so that the phenotype of GM-3C-2 cells carrying the *ANB1/CYC1* fusion on plasmid YCp*CYC1*(2.4)1/X is still one of complete respiratory deficiency, giving rise to negligible growth on lactate plates. The properties of this fusion provided an opportunity to select for mutants constitutive for expression of the *ANB1* gene. A mutation that interfered with the aerobic repression acting through the *ANB1* modulator would permit expression of the *ANB1/CYC1* fusion in the presence of oxygen and, consequently, allow growth on lactate plates.

GM-3C-2 cells carrying YCp*CYC1*(2.4)1/X were mutagenized with UV radiation or ethyl methanesulfonate and plated on lactate plates. Lactate-positive mutants were obtained at a frequency of $\approx 10^{-4}$. These displayed a wide range of growth rates on lactate plates.

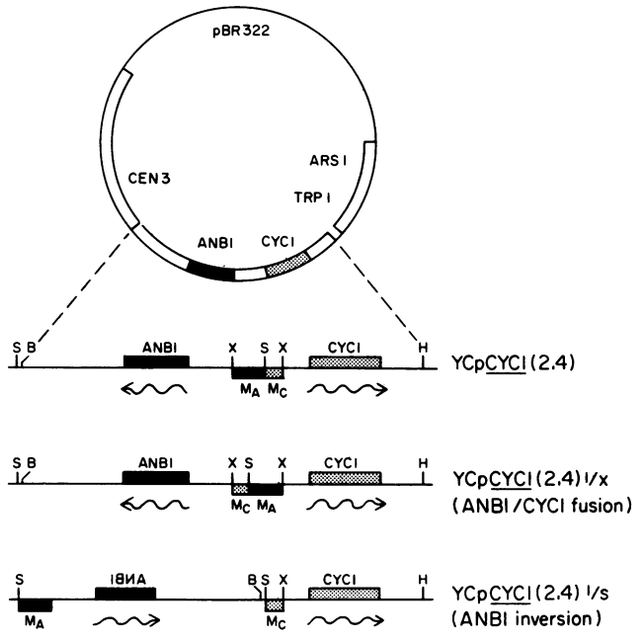

FIG. 1. Plasmids carrying the wild-type *ANB1* and *CYC1* genes, the *ANB1/CYC1* fusion, and the *ANB1* inversion. Construction of plasmids YCp*CYC1*(2.4) and YCp*CYC1*(2.4)1/X has been described (15). Plasmid YCp*CYC1*(2.4)1/S was constructed by digesting YCp*CYC1*(2.4) with *Sma* I, religating, and screening *E. coli* transformants for clones containing plasmid with the 1.6-kb *Sma* I fragment carrying the *ANB1* gene inserted in the reverse orientation. The transcriptional orientation of the two genes is indicated by the directional arrows for the different plasmid constructions. The elements M_A and M_C represent the modulator regions of the two genes, a pair of regions bounded by *Xho* I and *Sma* I sites, which contain regulatory sequences for the *ANB1* and *CYC1* genes, respectively. Restriction sites are indicated by the following abbreviations: S, *Sma* I; X, *Xho* I; B, *Bam*HI.

Discrimination of *cis*- and *trans*-Acting Constitutive Mutations. The mutagenesis was carried out on cells that had already been transformed with the fusion plasmid to obviate the necessity of transforming large numbers of cells after mutagenesis. The main purpose of the mutant selection was to obtain *trans*-acting mutations in chromosomal genes involved in regulation of *ANB1* expression, but it was expected that the lactate-positive isolates would also include some carrying *cis*-acting mutations within the YCp*CYC1*(2.4)1/X plasmid. Since all *cis*-acting mutants were expected to be dominant, they were tentatively screened for by mating with aRZ5 (*cyc1*, *cyc7*) and by testing the diploids for lactate-positive phenotype. Both dominant and recessive mutations were found; the former predominating after UV mutagenesis, the latter after ethyl methanesulfonate mutagenesis. In addition, some "semi-dominant" mutants were present, as revealed by slow but detectable growth of diploids on lactate plates. A more definitive *cis/trans* test involves curing the mutant of the plasmid it carries, retransforming with unmutagenized plasmid, and testing for mutant phenotype. Cells carrying *trans*-acting mutations will still constitutively express the *ANB1/CYC1* fusion (lactate$^+$), while cells that originally carried *cis*-acting mutations in the plasmid revert to wild-type expression (lactate$^-$). For this test, a group was selected, including dominant, semi-dominant, and recessive mutants. After curing and retransformation, these were now found to fall into three categories: *trans*-recessive, *trans*-semi-dominant, and *cis*-dominant. These last plasmid-borne mutations were found to be due to large deletions or inser-

tions and have not been studied in detail. A subset of the semi-dominant and recessive *trans*-acting mutants were selected for further characterization.

Genetic Analysis of Constitutive *trans* Mutations. After conversion of several mutant strains from *MATα* to *MATa* by transformation with the YEp*HO* plasmid (21), the appropriate isogenic *MATa* and *MATα* mutants were mated, and the resulting diploids were selected and tested for complementation. Complementation between mutants was shown by the restoration of wild-type regulation of the *ANB1/CYC1* fusion, apparent as a lack of growth on lactate.

Two complementation groups were found and were designated *rox1* and *rox2* (for *r*egulation by *ox*ygen). The *rox2* mutants, although expressing the *ANB1/CYC1* fusion, did not prove to be constitutive for *ANB1* expression (see below) and have not been studied in detail. We noted, however, that all these mutants showed flocculent growth morphology and that constitutive expression of the fusion was unstable, declining during maintenance on YPD agar.

The *rox1* mutants, which were stable and nonflocculent, fell into two classes: *rox1-a*, which are semi-dominant, and *rox1-b*, which are recessive. Diploids constructed between *rox1-a1* and various *rox1-b* mutants were much more strongly lactate-positive than diploids constructed between *rox1-a1* and the parent strain, indicating that the two groups were allelic. However, this lack of complementation was ambiguous because *rox1-a* mutants were semi-dominant. To confirm linkage, tetrad analysis was undertaken using diploids constructed from strains CL-2-15a and CL-1-13b, which carry the *rox1-a1* and *rox1-b3* mutations, respectively. These strains were derived from crosses between aRZ5 and the original *rox1-a1* and *rox1-b3* isolates. The diploids carried YCp*CYC1*(2.4)1/X, so that haploid segregants could be scored for constitutive expression of the *ANB1/CYC1* fusion. All of the segregants (total of 26) which still carried the YCp*CYC1*(2.4)1/X plasmid after meiosis showed the *rox1* phenotype, among haploids from six full tetrads and several partial tetrads, demonstrating linkage between the *rox1-a* and *rox1-b* classes of mutation. Taken together, the functional noncomplementation and genetic linkage of the phenotypically distinct *rox1-a* and *rox1-b* mutations strongly indicate that they are alleles of a single gene.

Phenotypic Analysis: Constitutive Expression of *ANB1*. According to the original design of the selection, mutants expressing the *ANB1/CYC1* fusion in YCp*CYC1*(2.4)1/X were expected to include some that would be constitutive for expression from the wild-type *ANB1* gene. To test for this property, mutants that had been cured of the YCp*CYC1*(2.4)1/X plasmid were retransformed with YCp*CYC1*(2.4), which carries the wild-type *ANB1* and *CYC1* genes. RNA from several aerobically grown isolates was subjected to RNA blot analysis. Fig. 2 shows a blot of some of these samples, probed with a fragment carrying the *ANB1* gene, which hybridizes both to the *ANB1* transcript and to another homologous transcript, *tr-1*. The *tr-1* gene (15) is located elsewhere in the genome and is positively regulated by oxygen, although it shares homology with *ANB1*. As shown, all the *rox1* mutants were constitutive for *ANB1* expression—i.e., they accumulated variable amounts of *ANB1* transcript in the presence of oxygen, whereas the *rox2* mutants were normally regulated.

Pleiotropic Effects: Constitutive Expression of Aerobic Genes in *rox1-a* but Not in *rox1-b* Cells. In wild-type cells, expression of a number of genes is induced by oxygen and is negligible under anaerobic conditions. Among these are three genes for which clones were available as probes for RNA blots: (*i*) *CYC1*, (*ii*) *tr-1*, and (*iii*) *SOD*, the superoxide dismutase gene. To examine the regulation of expression of these genes in *rox1-a*, *rox1-b*, and *rox2* mutants, representative strains transformed with the wild-type plasmid YCp*CYC1*(2.4) were grown anaerobically and aerobically in glucose, and aerobically on the catabolite derepressing sugar raffinose. RNA was extracted and analyzed by RNA blot, using a membrane that could be efficiently stripped and reprobed, permitting semi-quantitative comparison of several different transcripts in the same gel sample. *rox1-b*, *rox2*, and GM-3C-2 cells were all subject to strong oxygen induction of *tr-1* (Fig. 3*a*), *SOD* (Fig. 3*b*), and *CYC1* (Fig. 3*c*), evident by the increased levels in aerobic cultures compared to low or undetectable levels in anaerobic cultures (lanes 1 and 2, 4 and 5, and 10 and 11). In contrast, the *rox1a-1* mutant showed equal levels of the three transcripts in cells from glucose cultures grown under anaerobic and aerobic conditions (lanes 7 and 8). In the case of *CYC1* mRNA, aerobic levels were lower than those found in the parent strain under the same conditions, but they were easily detectable.

Catabolite repression of *CYC1* mRNA and *SOD* mRNA was observed in GM-3C-2 and in all the mutants tested, as shown by comparing RNA from the aerobic derepressed (raffinose) and repressed (glucose) cultures. However, the derepressed levels of *CYC1* mRNA in *rox1a-1* were again significantly lower than in GM-3C-2.

Pleiotropic Effects of *rox1-a1* Due to a Single Mutant Allele. One trivial explanation for the unusual pleiotropic effects of the *rox1-a1* mutation is that the isolate studied carries two mutations affecting anaerobic and aerobic genes independently. Since tight linkage of two independent nonallelic mutations would not be expected, a relatively reliable test of true pleiotropy was to follow the segregation of the two distinguishable regulatory phenotypes—constitutive expression of *ANB1* and of the aerobic genes. Spore analysis of diploids resulting from a cross of the *rox1-a1* and aRZ5 was done to determine whether the two regulatory phenotypes

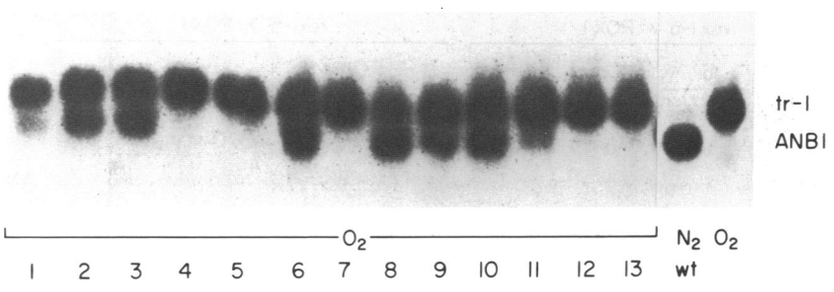

FIG. 2. *ANB1* and *tr-1* transcripts in mutants carrying YCp*CYC1*(2.4). *rox1* mutants numbered 1, 2, 3, 6, 8, 9, 10, and 11, and *rox2* mutants 4, 5, 7, 12, and 13 were transformed with YCp*CYC1*(2.4) and grown aerobically in YPD. RNA was extracted and subjected to RNA blot analysis using a nick-translated fragment [the 1.2-kb *Xho* I/*Bam*HI fragment from YCp*CYC1*(2.4)] carrying the *ANB1* gene as probe. The parental cells (wt; GM-3C-2) carrying YCp*CYC1*(2.4) were grown anaerobically and aerobically for comparison.

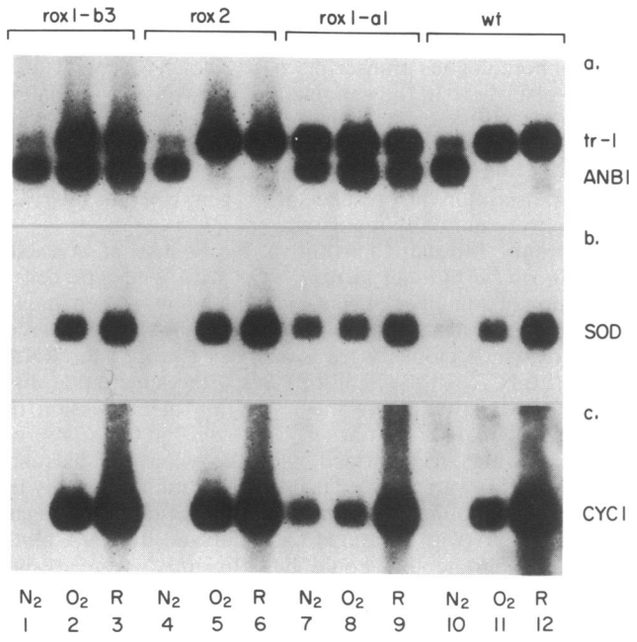

FIG. 3. Analysis of RNA in selected mutants grown under anaerobic, aerobic, and catabolite derepressed conditions. (*a*) RNA was extracted from mutant and wild-type (GM-3C-2) cells carrying YCp*CYC1*(2.4), grown as indicated: N_2, anaerobically in YPD; O_2, aerobically in YPD; R, aerobically in YPR (2% raffinose substituted for glucose). RNA was analyzed by RNA blot, using the same *ANB1* gene probe as in Fig. 2. (*b*) The same blot, stripped of probe and rehybridized with a nick-translated 1.2-kb *Bam*HI fragment carrying the *SOD* gene, derived from plasmid pFL1-cIII-FeS-3 (21). (*c*) The same blot, stripped of probe and rehybridized with the nick-translated pYe*CYC1*(0.6) plasmid (22), which carries most of the coding region of the *CYC1* gene.

cosegregated. Haploid segregants were dissected from six tetrads and grown under anaerobic and aerobic conditions. RNA extracted from these cells was analyzed on RNA blots by hybridizing successively with the *CYC1* gene and then with the *ANB1* gene. Although there was variation among the strains tested, in all cases segregants that were constitutive for *ANB1* expression were also constitutive for expression of the aerobic genes. Conversely, segregants that showed wild-type regulation for *ANB1* also expressed the aerobic genes normally. The linkage of the two *rox1-a1* phenotypes during meiosis indicates that the diverse regulatory effects are due to the presence of a single mutant allele. Fig. 4 shows an RNA blot of some of the samples that were analyzed. Tetrads obtained from crosses between aRZ5 and either the *rox1-a1* or *rox1-b3* mutants showed normal 2:2 segregation of *ROX1* alleles. The *rox1-a1* allele remained pleiotropic (as shown in Fig. 4 by constitutive expression of *tr-1* and *ANB1*, with their transcripts evident in anaerobic and aerobic cells, respectively). The *rox1-b3* allele continued to render only *ANB1* expression constitutive.

Joint Constitutivity of *ANB1* and *CYC1* Expression Does Not Require Proximity. It was previously found that wild-type regulation of the *ANB1* and *CYC1* genes is physically independent, in the sense that their respective regulatory regions can be separated without affecting expression (unpublished data). This was shown by analysis of the expression of *ANB1* and *CYC1* from the plasmid YCp*CYC1*(2.4)1/S (Fig. 1). In this construction, a *Sma* I fragment containing the *ANB1* gene and its modulator region was inserted in the reverse orientation, producing a separation of ≈1.6 kb between the *ANB1* and *CYC1* regulatory sites. Regulation in GM-3C-2 cells transformed with this plasmid was identical to that in cells transformed with the wild-type genes on YCp*CYC1*(2.4). Even though regulation of the two genes thus appeared to be physically independent in the parent strain, it still remained possible that the novel coconstitutive effect on *ANB1* and *CYC1* expression caused by the *rox1-a1* mutation was the result of interaction between a disturbed regulatory complex at the *CYC1* modulator and the proximal *ANB1* modulator. This was tested by an experiment in which RNA extracted from anaerobic and aerobic *rox1-a1* cells carrying the 1/S plasmid was compared to that obtained (Fig. 3 *a* and *c*, lanes 7–9) from *rox1-a1* cells carrying the wild-type plasmid YCp*CYC1*(2.4). An identical pattern (not shown) of constitutive expression was observed, indicating that the pleiotropic effect does not require physical proximity, but rather involves a common *trans*-acting element.

DISCUSSION

The isolation of mutants constitutive for expression of the anaerobic *ANB1* gene has revealed the existence of a *trans*-acting factor, the *ROX1* gene product, which is involved in regulation of *ANB1* and a number of oxygen-induced genes. This indicates that there is a generalized coordinate regulation of oxygen-sensitive genes effected, at least in part, by a common mechanism.

The *ROX1* gene product might act at any of several different levels: (*i*) control of transcription, (*ii*) post-transcriptional modification, (*iii*) specific breakdown of mRNA, (*iv*) translational control, and (*v*) production of a regulatory effector. However, by process of elimination, a direct role in regulation of transcription appears the most likely.

The third and fourth possibilities are argued against by a comparison between the expression of wild-type and fused genes. That is, the fact that *rox1* mutations cause constitutive expression of both the *ANB1* gene and the *ANB1/CYC1* fusion, but not of the normal *CYC1* gene, indicates that *ROX1*-mediated regulation of expression is independent of the nature of the transcript, being effected through upstream

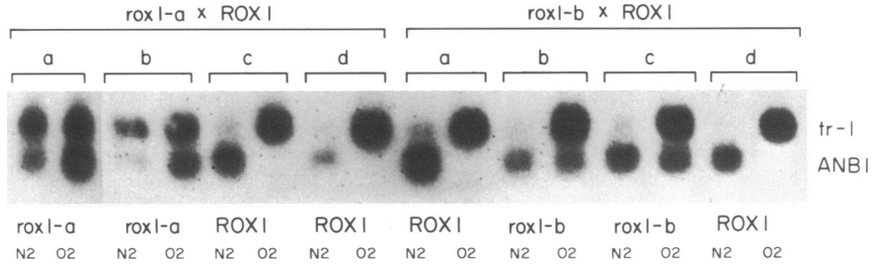

FIG. 4. Tetrads analyzed for expression of *ANB1* and *tr-1*. Mutants *rox1-a1* and *rox1-b3* carrying YCp*CYC1*(2.4) were mated with aRZ5, diploids were sporulated, and asci were dissected. Segregants carrying YCp*CYC1*(2.4) were grown anaerobically or aerobically in YPD. RNA was extracted and analyzed by RNA blot, using the same *ANB1* gene probe as in Fig. 2. The genotypes inferred from the regulation of expression are shown as *rox1* or *ROX1*.

control sequences instead. Therefore, since regulation at the level of mRNA translation or degradation would be presumed to be mRNA sequence-specific, participation by the *ROX1* gene product in either process is unlikely. Control at the level of post-transcriptional processing is a possibility; however, there is no evidence of a higher molecular weight precursor.

Perhaps the most likely alternative to a role in transcription is the fifth possibility, that the *ROX1* gene product is involved in the metabolism of a regulatory effector. However, it is difficult to explain the pleiotropic effects of the *rox1-a1* mutation in terms of changes in effector concentrations, because the mutation causes constitutive expression of both anaerobic and aerobic genes. In wild-type cells, these genes are normally regulated in opposite ways by oxygen levels, so that their expression would either have to respond in opposite ways to a common effector or else be controlled by different effectors. In either case, a mutation causing a change in the level of a single effector would not be expected to cause the observed increased expression of both types of genes. Hence, mutational changes in effector levels cannot account in a simple way for the pleiotropic effect on the expression of these oppositely regulated genes, although more complex metabolic explanations can still be considered.

The preceding argument favors a role for the *ROX1* gene product in regulation of transcription of oxygen-induced and oxygen-inhibited genes. Assuming that the *ROX1* gene product interacts with, or forms a part of, the transcriptional apparatus, a number of plausible explanations for the observed pleiotropic effects of *rox1* mutations can be proposed. The two simplest ones invoke a dual role for the *ROX1* factor in regulation of the two classes of genes. Both models are consistent with the occurrence of pleiotropic and nonpleiotropic mutant alleles of *ROX1*. In one model, the factor would activate one class of genes or the other in response to different effectors present in aerobic or anaerobic cells (or in response to the presence or absence of a single effector). In an alternative bimodel mechanism, the *ROX1* factor would simultaneously participate in repression of *ANB1* and activation of the oxygen-induced genes when complexed with oxygen-induced effector. The possibility that a single factor can function as a part of either an activating or a repressing complex with different genes has a precedent in the versatile role suggested for the *a1* component of the mating-type control system (21).

It is not possible to conclude at present whether the control of expression mediated by the *ROX1* gene product is positive or negative. An inference based on the dominance or recessiveness of regulatory mutations is clearly not possible, because the *rox1* mutations include both recessive and semidominant alleles. The properties of true null mutations will be informative in this regard.

Possible Consensus Sequences for Oxygen-Regulated Genes. The pleiotropic regulatory phenotype of the *rox1-a1* mutation provides evidence of a common mechanism controlling expression of a group of oxygen-regulated genes. It might be expected that there would be sequence homology between the regulatory sites of the coordinately controlled genes. In fact, a striking homology has been found between DNA segments in the upstream regions of three oxygen-regulated genes, *CYC1* (4), *SOD* (L. Grivell, personal communication), and *CYC7* (26), a finding that supports the hypothesis of a common regulatory complex acting through these sites.

The upstream region of the *ANB1* gene contains no discernible homology to the segments alluded to above. This suggests that a different recognition sequence may be involved in *ROX1*-mediated regulation of *ANB1* expression, which could help account for the opposite response to oxygen in the expression of the anaerobic and aerobic genes.

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